

DISSERTATION

COMPLEX EVOLUTION OF MITOCHONDRIAL GENOMES:
LESSONS FROM SALAMANDERS

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ABSTRACT

COMPLEX EVOLUTION OF MITOCHONDRIAL GENOMES: LESSONS FROM SALAMANDERS

This dissertation research on genome evolution focuses on understanding the mechanisms that drive the evolution of mitochondrial genome size, content, and organization in animals. This research uses a combination of empirical and computational approaches to examine the evolution of mitochondrial genomes in vertebrates, focusing on salamanders as an exemplar clade.

Chapter One analyzes mitochondrial genome sequences of vertebrate lineages that differ in metabolic rates. Salamanders, which have the lowest metabolic requirements among tetrapods, experience weaker purifying selection on protein-coding sequences than do frogs, a comparable amphibian clade with higher metabolic rates. In contrast, there is no evidence for weaker selection against mitochondrial genome expansion in salamanders. Together, these results suggest that different aspects of mitochondrial genome evolution (i.e. nucleotide substitution, accumulation of non-coding sequences) are differently affected by metabolic variation across tetrapod lineages.

Chapter Two describes the correlation between gene rearrangement/genome expansion and increased rates of substitution in salamander mitochondrial genomes. Most protein-coding genes maintain their position along the mutation gradient in rearranged/expanded mitochondrial genomes, and the genes that do move are unaffected by their new position because the mutation gradient in salamanders is weak. Additionally, genomic rearrangements/expansions occur independent of levels of selective constraint on genes. Together, these results demonstrate that

large-scale changes to genome architecture impact mitochondrial gene evolution in predictable ways; however, despite these impacts, the same functional constraints act on mitochondrial protein-coding genes in both modified and normal genomes.

Chapter Three reports the phylogenetic relationships among lineages of *Aneides*, sampling both within and among all six species, based on three nuclear markers and describes mitochondrial genome sequences for nine of the taxa represented in the phylogeny. Mitochondrial gene order and level of mitochondrial sequence divergence were estimated for these sequences and two previously published sequences. Two genome duplication events resulting in mitochondrial gene rearrangements were detected, the first rearrangement occurring in the common ancestor of *Aneides* and the second rearrangement existing across different populations of a single species, *A. hardii*. Comparisons of *hardii* genomes show that duplicated protein-coding and rRNA genes are lost more rapidly than other duplicated mitochondrial sequence (i.e. tRNAs, non-coding sequence) and suggests that these large scale changes can occur across very shallow levels of genetic divergence.

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INTRODUCTION

Understanding the evolutionary processes that shape the size and complexity of eukaryotic genomes (mitochondrial, chloroplast, and nuclear) is a fundamental goal in biology. Recent advancements in sequencing technology and computational methods bring evolutionary biology fully into the post genome era. Specifically, the accessibility of obtaining genome-scale data, even for non-model systems, has made it possible to learn about genomes as whole entities and is transforming the way we think about genomes, revealing them as complex and dynamic. Additionally, new comparative genomic approaches show us how genomes can differ significantly in both structure and content, raising questions about what evolutionary processes shape genome diversity. Collectively, these factors have revitalized the field of genome evolution.

Mitochondrial genomes are found within the mitochondria, which are the site for the citric acid cycle and oxidative phosphorylation (OXPHOS), the final steps of ATP synthesis via cellular respiration. Each mitochondrion contains multiple copies of its own genome, which in animals is generally a small circular DNA molecule (~17 kb in vertebrates (Moritz, et al. 1987; Boore 1999; Scheffler 2008)). The mitochondrial genome is usually comprised of 13 genes, which encode subunits of the protein complexes that perform electron transport and ATP synthesis, as well as 22 tRNAs and 2 rRNAs that are required for transcription and translation of these genes within the mitochondrion (Moritz, et al. 1987; Boore 1999; Scheffler 2008). Because of its small size, the mitochondrial genome has served as an important tractable model for examining the relationships among the different forces shaping the evolution of genes and genomes.

Differences in the mitochondrial genome may significantly impact organismal phenotype. Studies are beginning to show that differences in metabolic demand across tetrapod lineages impact mitochondrial genome evolution (Castoe, et al. 2008; Shen, et al. 2009; Shen, et al. 2010); however, our understanding of the underlying mechanisms still remains unclear. For my dissertation research, I am investigating how the interplay between mutation, selection, and drift impacts mitochondrial genome evolution across species. Specifically, I am (1) testing for differences in selective constraint on mitochondrial genes associated with differences in metabolic requirements, (2) characterizing the mutational processes underlying mitochondrial genome size and content variation, and (3) evaluating the effects of gene duplications on mitochondrial genome evolution.

To examine the role of mutation, selection, and genetic drift in driving mitochondrial genome evolution, I conducted three studies using a combination of empirical and computational approaches focusing on salamanders as an exemplar clade. Salamanders exhibit variation in mitochondrial genome size and organization among close relatives (Mueller and Boore 2005; Mueller 2006), which provides an ideal system in which to examine how non-adaptive processes (i.e. mutation and drift) and natural selection interact to shape genome size and complexity. For my first study, I tested for differences in selective constraint on mitochondrial genes and differences in mitochondrial genome size between salamanders and frogs, a comparable amphibian clade with higher metabolic rates (Gatten, et al. 1992). I also examined the relationship between mitochondrial genome rearrangements/expansions and substitution rates in mitochondrial genes, focusing on genomic comparisons within salamanders, to test if a positive correlation exists across the mitochondrial genome as has been documented in other taxa (Shao, et al. 2003; Broughton and Reneau 2006; Xu, et al. 2006). I then quantified differences in

selective constraint on mitochondrial genes between normal and rearranged/expanded genomes. Lastly, I use a genus of salamander in which mitochondrial gene rearrangements have been documented (Mueller and Boore 2005) to determine the origins of mitochondrial genome duplication events and examine the evolutionary fate of duplicated mitochondrial genes. My dissertation research is among the first to exploit a study system where highly variable levels of selective constraint across OXPHOS functional complexes allow a rigorous exploration of the impacts of mutation and the effects of functional constraint on genome evolution and how these factors shape genome diversity.

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CHAPTER 1

LOW METABOLIC RATES IN SALAMANDERS ARE CORRELATED WITH WEAK SELECTIVE CONSTRAINTS ON MITOCHONDRIAL GENES

INTRODUCTION

Mitochondria are the site for the citric acid cycle and oxidative phosphorylation (OXPHOS), the final steps of ATP synthesis via cellular respiration. In addition to their critical role in meeting the cell's continuous energy demands, mitochondria also perform a variety of other functions associated with cellular Ca^{2+} signaling, apoptosis, cell transport, thermoregulation, and immunity (Bonawitz et al. 2006a; Bonawitz et al. 2006b; Detmer and Chan 2007; Scheffler 2008). Each mitochondrion contains multiple copies of its own genome, generally a small circular DNA molecule (~17 kb in vertebrates) (Boore 1999; Scheffler 2008). Although mitochondria themselves perform diverse tasks, the mitochondrial genome encodes proteins associated with only one key task — OXPHOS. The mitochondrial genome usually comprises 13 genes that encode subunits of the protein complexes that perform electron transport and ATP synthesis, as well as 22 tRNAs and 2 rRNAs for transcription and translation of these genes within the mitochondrion (Boore 1999; Scheffler 2008). Nuclear genes encode the remaining 76 protein subunits required for OXPHOS (Ryan and Hoogenraad 2007; Scheffler 2008). Thus, metabolic requirements constrain substitution patterns of the entire mitochondrial genome, as well as a subset of the nuclear genome, to maintain the functional integrity of OXPHOS machinery.

Mitochondria exist not as discrete organelles, but as part of a dynamic network characterized by fission, fusion, and intra-cellular transportation events triggered in response to

cellular stimuli (Detmer and Chan 2007). Mitochondrial network organization impacts all mitochondrial functions. Thus, functional OXPHOS proteins are necessary, but not sufficient, to provide cells with ATP; dynamic control of the mitochondrial network in response to cellular energy demands is also required (Benard et al. 2007). Such control results from the coordinated expression of ~1500 proteins that make up the mitochondrial proteome (Gibson 2005; Scheffler 2008). The vast majority of these genes are encoded by the nuclear genome, translated in the cytosol, and imported into the mitochondria (Neupert and Herrmann 2007). However, regulation of both mitochondrial transcription and mitochondrial DNA replication is also a critical component of this coordinated gene expression and network control (Fernandez-Silva et al. 2003; Bonawitz et al. 2006a). Mitochondrial genes are transcribed as polycistronic transcripts that span almost the entire length of the genome, and mitochondrial DNA replication proceeds from a single replication origin per strand. Thus, both RNA transcription and DNA replication rates are likely impacted by mitochondrial genome size, which varies across the Tree of Life (Rand 1993). Constraints imposed by mitochondrial network organization, critical for both OXPHOS and non-OXPHOS functions, may therefore shape the evolution of mitochondrial genome size, although this hypothesis remains untested.

Among metazoans, average rates of substitution are higher in the mitochondrial genome than in the nuclear genome (Brown et al. 1979), reflecting the highly mutagenic mitochondrial environment (Ballard and Dean 2001; Scheffler 2008; Santos 2012). Coalescent times are also shorter because of the smaller haploid effective population size (Moritz et al. 1987; Ballard and Rand 2005). Mitochondrial sequences have therefore proven to be a powerful tool for evolutionary analyses. Although such studies have often assumed that mitochondrial genome evolution is effectively neutral across lineages (Ballard and Kreitman 1995; Blier et al. 2001),

the mitochondrial genome's central role in ATP synthesis, coupled with the large-scale differences in aerobic metabolic rates across taxa, suggests that this assumption may be invalid (Ballard and Kreitman 1995; Bazin et al. 2006; Galtier et al. 2009b; Rand 2011). Consistent with this prediction, recent studies have begun to examine how organismal differences in key traits linked to mitochondrial biology (particularly aerobic metabolic rate, but also temperature) are reflected in different selective regimes acting on the mitochondrial genome (Galtier et al. 2009a; Sun et al. 2011). For example, studies in birds and mammals have tested for differences in selective constraints on mitochondrial DNA sequences across groups that differ in locomotive ability, a proxy for metabolic demand (Shen et al. 2009). These studies show that more weakly locomotive organisms experience weaker purifying selection on metabolic genes. Additionally, Shen et al. (Shen et al. 2010) identified positive selection on mitochondrial genes along the lineage leading to bats, suggesting adaptive evolution to meet the increased energy demands of flight. Finally, Castoe et al. (Castoe et al. 2008) identified extensive positive selection on mitochondrial genes in the ancestral snake lineage, suggesting adaptive evolution for extreme metabolic flexibility. Convergent with snakes, a similar pattern of molecular evolution in mitochondrial genes was detected in agamid lizards (Castoe et al. 2009). These studies provide the first steps towards exploring how differences in metabolic demand across tetrapod lineages impact the strength and type or selection acting on the mitochondrial genome; however, the full range of metabolic demands that characterize different lineages remains unexplored.

Salamanders have the lowest metabolic requirements among tetrapods (Gatten et al. 1992). However, the impacts of such low metabolic rates on mitochondrial genome evolution remain unexplored. In this study, we show that the salamander clade experiences relatively weak purifying selection on mitochondrial gene sequences across all four OXPHOS functional

complexes. In contrast, we find no evidence for relatively weak selection against mitochondrial genome expansion in salamanders. Taken together, these patterns suggest that different aspects of mitochondrial genome evolution (i.e. nucleotide substitution in gene sequences, accumulation of non-coding sequences) are differentially affected by variable metabolic requirements across tetrapod lineages.

METHODS

Taxon selection and datasets. – We chose to compare salamanders with frogs (their sister taxon within the lissamphibia) because these two ectothermic amphibian clades share similar thermal habitats and experience similar environmentally dictated body temperatures. Although variation in metabolic rate exists among lineages within both clades, frog resting metabolic rates are, on average, 1.5- to 2.5-fold higher than those of salamanders (Figure 1.1, Table A1.1), and minimal cost of transport is significantly lower in salamanders (Feder 1976; Gatten et al. 1992). These differences suggest that the frog and salamander clades have diverged in factors controlling metabolic rate since their point of common ancestry. The mechanistic explanation for this divergence remains unknown, although hypotheses include (1) differences in muscles (e.g. the energetic cost of muscular force production), as well as (2) salamanders' enormous nuclear genomes and associated low cell-surface-to-volume ratios (Szarski 1983; Taylor 1985; Kozlowski et al. 2003). Here, we test whether this metabolic divergence between clades (whatever its underlying mechanism) is correlated with a clade-level difference in selective constraints on mitochondrial genome sequences, which are directly associated with meeting an organism's metabolic requirements. We emphasize that we are not evaluating whether metabolic rate and selective constraint on mitochondrial genomes have remained correlated throughout the

evolutionary history of anuran and caudate amphibians, a different question that would require regression analyses controlling for phylogenetic non-independence (e.g. independent contrasts, phylogenetic generalized least squares) (Santos 2012). Rather, we are evaluating whether a historical divergence in metabolism between these two clades has had long-lasting, persistent consequences for molecular evolution, a pattern that would be detectable by comparing characteristics of any subsets of extant lineages between the two clades.

We obtained whole mitochondrial genome sequences and partial mitochondrial genomes (i.e. sequences that contain all of the protein-coding genes, but lack a portion of the non-protein-coding sequence) for 94 salamanders (53 whole, 41 partial) and 34 frogs from GenBank (Table A1.2). Our total dataset includes representatives of all 10 salamander families and 12 of 38 frog families that encompass the basal/near-basal split within frogs (Roelants et al. 2007) (Figure 1.1).

To allow for comparisons between the mitochondrial genome and nuclear (i.e. non-metabolic) sequences, we also obtained sequences for four nuclear genes from diverse salamander and frog lineages chosen to maximize taxonomic overlap with the mitochondrial dataset: BDNF for 46 salamanders and 30 frogs, NCX1 for 23 salamanders and 33 frogs, POMC for 48 salamanders and 33 frogs, and RAG1 for 54 salamanders and 40 frogs (Table A1.2). Of the 52 genera represented in our salamander mitochondrial dataset, 27 genera are represented by one or more species of the same genus in our BDNF data, 18 genera are represented in our NCX1 data, 28 genera are represented in our POMC data, and 34 genera are represented in our RAG1 data. Of the 21 genera of frog represented in our mitochondrial dataset, 15 genera are represented by one or more species of the same genus in our BDNF data, 14 genera are represented in our NCX1 data, 15 genera are represented in our POMC data, and 19 genera are represented in our RAG1 data.

Alignment and initial phylogenetic analysis. – We performed multiple sequence alignments based on amino acid sequences for each gene (13 mitochondrial and 4 nuclear) using MUSCLE v.3.8 (Edgar 2004). We excluded regions of ambiguous alignment, based on the presence of indels, from further analysis. We then estimated ten maximum likelihood (ML) trees: (1) the concatenated mitochondrial tree for salamanders, (2) the concatenated mitochondrial tree for frogs, (3-4) the BDNF trees for salamanders and for frogs, (5-6) the NCX1 trees for salamanders and for frogs, (7-8) the POMC trees for salamanders and for frogs, and (9-10) the RAG1 trees for salamanders and for frogs. All of these trees were estimated using RAxML v.7.2 (Stamatakis 2006), partitioning the data by gene and codon position and specifying the GTR + Γ model of nucleotide substitution for each partition. These ten trees serve as the basis for our selection analyses.

Selection Analyses. – To test whether salamanders experience weaker purifying selection on their mitochondrial genes than do frogs, we compared the ratio of nonsynonymous (dN) to synonymous (dS) substitution rates ($\omega = dN/dS$) between the two clades. ω is commonly used to measure the strength of selection: a small ω value indicates strong purifying selection and a large ω value (where ω is still less than 1) indicates weak, or relaxed, purifying selection.

For each mitochondrial gene, we first estimated dN, dS, and ω for both salamanders and frogs assuming a single value for each parameter across all branches (Model 0 in Codeml, implemented in PAML v4.4 (Yang 2007)); topologies were fixed to the ML trees estimated from the concatenated mitochondrial sequences. We then used these estimates as starting values to estimate dN, dS, and ω under a model that allows these parameter values to vary for each branch on a tree (Model 1 in Codeml). Although this analysis yields ω estimates for all branches (both internal and tip), we restricted one round of comparisons to tip taxon estimates because these are

less likely to be impacted by uncounted multiple substitutions at any given site. We then repeated our analyses including both internal and tip branches to ensure that any patterns detected were not restricted to tip lineages. For each gene, we tested for differences in ω values between salamanders and frogs using a Mann-Whitney test implemented in R, as the data were non-normally distributed (Shapiro-Wilks test for normality, $p < 0.0001$). Additionally, we performed ten replicate sub-analyses under equal taxon sampling (i.e. randomly selecting 34 of our 94 total salamanders) for each gene to test whether any differences detected in ω between the two clades reflected bias introduced by having more salamander than frog sequences in our dataset. Finally, we performed the same set of analyses on all four nuclear genes to exclude the possibility that any difference in ω between salamander and frog mitochondrial genes reflects differences in the strength of genetic drift between the two clades. Stronger genetic drift, as a result of smaller effective population size, would lead to higher ω values for genes encoded by both genomes, irrespective of metabolic function.

Genome Size Analysis. – If salamanders experience weaker selection for DNA replicational and RNA transcriptional efficiency than do frogs, we would predict a pattern of larger mitochondrial genomes (i.e. the sequence length of complete genomes) in salamanders than in frogs (Selosse et al. 2001). We tested for a difference in overall mitochondrial genome size between the two clades using a Mann-Whitney test implemented in R, as the data were non-normally distributed (Shapiro-Wilks test for normality, $p < 0.0001$).

RESULTS AND DISCUSSION

Selection. – ω is significantly larger in salamanders than in frogs for 10 of the 13 mitochondrial protein-coding genes (two-way Mann-Whitney test; $p < 0.0001$ for all 10 genes)

(Figure 1.2); no difference in ω between the two clades exists for the remaining three mitochondrial genes (ATP8, COX1, and COX2; $p > 0.31$ for all three genes). This pattern holds, both for our complete dataset, as well as the subsampled datasets controlling for unequal taxon sampling (two-way Mann-Whitney with Bonferroni correction for multiple comparisons). In contrast, ω for BDNF, POMC, and RAG1, nuclear-encoded genes not associated with metabolic function, are not significantly different between frogs and salamanders (two-way Mann-Whitney test, $p > 0.25$ for all three genes), and ω for the non-metabolic nuclear NCX1 gene is actually larger in frogs (two-way Mann-Whitney test, $p < 0.02$). Inclusion of only tip branches (Figure 1.2), or both internal and tip branches (Table A1.2), yields similar results. These estimates of ω for the nuclear genes suggest that the differences in ω between frog and salamander mitochondrial genes do not reflect varying strengths of genetic drift between the two clades. Taken together, our results are consistent with weaker purifying selection on mitochondrial genes in salamanders than in frogs, as predicted by clade-level differences in metabolic requirements. Our results are in agreement with similar analyses performed in birds and mammals; Shen et al. (Shen et al. 2009) demonstrate that mitochondrial ω values are lower in “strongly locomotive” lineages, which suggests stronger purifying selection associated with higher metabolic requirements. Thus, similar patterns emerge from comparisons involving highly aerobic, endothermic vertebrates (birds and mammals) as well as less aerobic, ectothermic vertebrates (salamanders and frogs).

The three genes that show no difference in ω between salamanders and frogs (ATP8, COX1, and COX2) fall on the opposite ends of the range of nonsynonymous substitution rates (dN) exhibited by mitochondrial genes. Across diverse vertebrate lineages, including those examined in this study, COX1 and COX2 have the lowest dN , while ATP8 has the highest

(Pesole et al. 1999; Shen et al. 2009). COX1, COX2, and COX3, in combination with ten nuclear-encoded protein subunits, form Complex IV (cytochrome *c* oxidase) of the electron transport chain; ATP6 and ATP8, along with fourteen nuclear-encoded subunits, form Complex V (ATP synthase). Our results suggest that the difference in functional constraint experienced by frog and salamander mitochondrial genes varies among the OXPHOS functional complexes. For the most constrained complex (Complex IV), the lower metabolic demands of salamanders may not translate into weaker purifying selection across all three genes; the ATP requirements of salamanders likely still impose the same level of selective constraint on COX1 and COX2 sequence evolution. For the least constrained mitochondrial protein (ATP8 of Complex V), high levels of dN (relative to other mitochondrial genes) may be permitted by the metabolic requirements of either clade. For the other two complexes (Complex I, composed of ND1-6 and ND4L and 36 nuclear-encoded proteins, and Complex III, composed of CYTB and ten nuclear-encoded proteins), the metabolic differences between salamanders and frogs likely translate into different degrees of purifying selection on all mitochondrial-encoded subunits. Similar analyses comparing strongly and weakly locomotive birds showed significant differences in ω for only four out of 13 mitochondrial genes, although analyses binning genes by functional complex showed differences in both Complex I and V (Shen et al. 2009). Thus, studies in amphibians and birds both suggest heterogeneous responses to variable metabolic constraint at the level of individual mitochondrial genes and functional complexes. Future studies will show whether such patterns hold for nuclear encoded OXPHOS proteins, as well as whether co-evolution between the two genomes is correlated with metabolic requirements.

Genome Size. – Many of the larger genomes in both salamanders and frogs result from repetitive sequences in the control region (the non-coding sequence that regulates replication and

transcription) and/or the presence of a duplicate control region (e.g. (Mueller and Boore 2005; Kurabayashi et al. 2010). Gene rearrangements resulting from tandem duplication and random loss are also present in both clades (e.g. (Mueller and Boore 2005; Kurabayashi et al. 2006; Kurabayashi et al. 2008). Our results show that mitochondrial genomes are not larger in salamanders than in frogs; in fact, frog genomes are larger (frog mean = 18.2 kb; salamander mean = 17.4 kb) (two-way Mann-Whitney test, $p < 0.0001$). Thus, we do not detect the pattern predicted by weaker selection against genomic expansion accompanying lower metabolic requirements in salamanders (Selosse et al. 2001). Instead, non-adaptive processes including genetic drift and/or a replicative advantage of genomes with duplicate control regions (irrespective of any consequences on organismal phenotype) may determine mitochondrial genome size variation in frogs and salamanders, as they do in other taxa (Kumazawa et al. 1998; Yokobori et al. 2004; Lynch et al. 2006; Boussau et al. 2011).

FIGURES

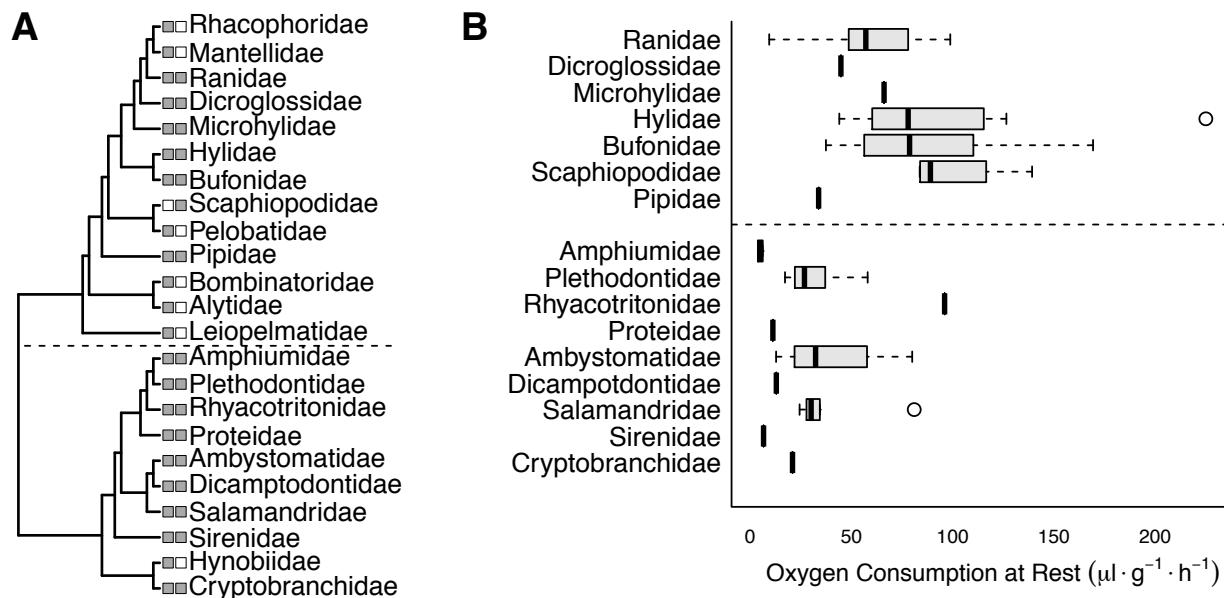


Figure 1.1 (A) Relationship of families used in this study and summary of taxa represented in the mitochondrial genome size dataset (first column) and the resting metabolic rate dataset (second column). The color of the box indicates the presence (grey) or absence (white) of data for a given family for each dataset. (B) Summary of published resting metabolic rates for families included in the genetic datasets. Frogs (top) and salamanders (bottom) are separated by the dashed line. Within both the salamander and frog clades, there is substantial overlap among families in metabolic rates; less overlap exists between salamanders and frogs.

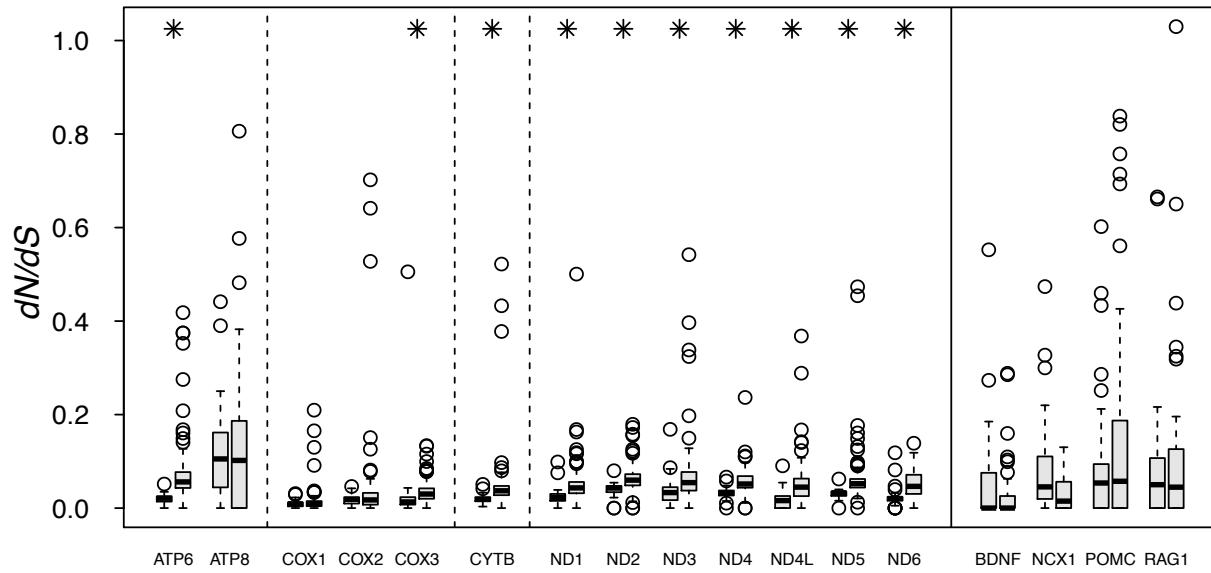


Figure 1.2 Comparison of dN/dS (ω) values for 13 mitochondrial protein-coding genes and four nuclear genes (BDNF, NCX1, POMC, and RAG1). Frogs are on the left; salamanders are on the right. Dashed lines separate mitochondrial genes belonging to different OXPHOS functional complexes, and the solid line separates mitochondrial and nuclear genes. For all mitochondrial genes except COX1, COX2, and ATP8, salamander ω values are significantly larger than frog values.

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CHAPTER 2

EVOLUTION ALONG THE MUTATION GRADIENT IN THE DYNAMIC MITOCHONDRIAL GENOME OF SALAMANDERS

INTRODUCTION

Mitochondria are intracellular organelles where oxidative phosphorylation (OXPHOS) is carried out to complete the process of ATP synthesis via cellular respiration. These organelles have their own genome (retained from their alpha-proteobacterial ancestor), which over evolutionary time has experienced extensive gene transfer to the nucleus (Andersson and Kurland 1998; Gray et al. 2001; Adams and Palmer 2003). In metazoans, this streamlining process has resulted in a small, circular DNA molecule encoding 13 peptides essential for electron transport, 22 transfer RNAs, and two ribosomal RNAs (Scheffler 2008). In addition, the mitochondrial genome also contains a control region (CR), a noncoding region that contains a replication origin and transcriptional promoter (Boore 1999; Saccone et al. 1999; Gissi et al. 2008; Scheffler 2008). Because of its small size, the mitochondrial genome has served as an important tractable model for examining the relationships among the different forces shaping the evolution of genes and genomes. Such forces include point mutations, larger mutations impacting genome size and architecture, selection on protein function, selection on transcriptional and translational efficiency, and genetic drift (Moritz et al. 1987; Rand 1993; Ballard and Dean 2001; Fernandez-Silva et al. 2003; Rand et al. 2004; Lynch et al. 2006; Detmer and Chan 2007; Scheffler 2008; Galtier et al. 2009; Boussau et al. 2011). Despite much progress, many important questions remain unanswered about how these forces interact to drive molecular evolution in mitochondrial genomes.

Although they have evolutionarily conserved genomic content, metazoan mitochondrial genomes show a diversity of gene orders (Boore and Brown 1998; Boore 1999; Saccone et al. 1999; Xu et al. 2006). Rearrangement of gene order, often accompanied by expansion of non-coding regions, results from gene duplications followed by random loss of one of the paralogs (Moritz and Brown 1987; Boore 1999; Mueller and Boore 2005; San Mauro et al. 2006) or by intramolecular recombination (Stanton et al. 1994). Variation in gene order is substantial across invertebrates, but is far less common in vertebrates (Boore 1999; Scheffler 2008). The reasons why some clades have more dynamic genomes than others remain unknown. The high rates of gene rearrangement in invertebrate mitochondrial genomes have been positively correlated with rates of nucleotide substitution within mitochondrial genes in mollusks, insects, crustaceans, and other arthropods (Hoffmann et al. 1992; Hoeh et al. 1996; Shao et al. 2003; Xu et al. 2006). The relationship between gene rearrangement and substitution rate is unexplored in vertebrates, and the mechanisms underlying this correlation remain unknown across all taxa.

In addition to variation in substitution rates across taxa associated with gene rearrangements, mitochondrial genes in some taxa also show spatial variation in substitution rates around the genome due to the mechanism underlying mitochondrial DNA (mtDNA) replication. Vertebrate mitochondrial replication initiates from two replication origins that are offset from each other, causing asynchronous replication across both strands (Figure 2.1). Specifically, mtDNA replication begins at the H-strand replication origin (O_H), displacing one strand and leaving it single-stranded, until the newly synthesized strand reaches the L-strand replication origin (O_L) and synthesis begins in the reverse direction along the displaced strand. Because single-stranded DNA is prone to mutation, mtDNA replication results in a gradient of mutation accumulation where regions that persist in the single-stranded state for longer

experience more AT-biased mutations (Reyes et al. 1998; Faith and Pollock 2003; Krishnan et al. 2004; Broughton and Reneau 2006; Scheffler 2008). The mitochondrial environment is mutagenic, in part, because of the presence of reactive oxygen species (ROS), byproducts of oxidative phosphorylation (Scheffler 2008). Metabolic rates vary dramatically across vertebrates, which may, in turn, yield different levels of mutagenicity, impacting the strength of the mutation gradient (Gatten et al. 1992; Santos 2012). To date, however, (1) the strength of the mutation gradient, (2) whether the mutation gradient produces a substitution rate gradient, and (3) whether genes in rearranged and/or expanded genomes move to more or less vulnerable positions along the gradient remain unexplored in the majority of vertebrates.

Substitution rates in mitochondrial genes are also shaped by functional constraints on mitochondrial proteins, which differ across both genes and lineages; these differences are associated with variation in organismal traits (Martin and Palumbi 1993; Rand 1994; Galtier et al. 2009; Sun et al. 2011). For example, high metabolic energy demands in birds and mammals are positively correlated with strong selective constraint (i.e. low levels of nonsynonymous relative to synonymous substitutions) on mitochondrial genes, whereas low metabolic demand in salamanders is correlated with weaker selective constraint (i.e. higher levels of nonsynonymous substitutions) (Shen et al. 2009; Chong and Mueller 2013). It remains unclear whether organisms experiencing weaker selection on mitochondrial gene sequences, which underlie OXPHOS protein function, also experience weaker selection on genome architecture (i.e. genome size and order), which impacts OXPHOS protein transcription and translation.

Salamanders, an amphibian clade of 648 species, include both “normal” mitochondrial genomes that have the vertebrate consensus mitochondrial gene order and a range of independently derived, modified genomes that have both gene order rearrangements and genome

expansions. Because this genomic diversity is unusual among vertebrates, salamanders provide a rare opportunity to test the effects of genomic modification on vertebrate mitochondrial gene sequence evolution. In this study, we show that (1) mitochondrial protein-coding genes within modified salamander genomes have significantly higher synonymous and nonsynonymous substitution rates than genes within normal salamander genomes. (2) Despite expansions of up to 6 kb, most genes in modified salamander genomes maintain their position along the mutation gradient. The genes that do move are not substantially impacted by their new position; the mutation gradient in salamanders is weak. (3) Gene rearrangements and genomic expansion events occur independent of levels of selective constraint acting on mitochondrial genes. Taken together, our results demonstrate that large-scale changes to genome architecture impact mitochondrial gene sequence evolution in predictable ways within salamanders; however, despite these impacts, the same functional constraints are acting on mitochondrial protein-coding genes in both modified and normal salamander genomes.

METHODS

Sequence data and genome characteristics

We obtained 62 complete salamander mitochondrial genome sequences, each representing a unique species, from the GenBank RefSeq database (release 53). Our dataset includes representatives from six of the 10 salamander families, with predominant taxonomic sampling representing the largest family, Plethodontidae. We extracted all 13 protein-coding genes from each mitochondrial genome based on GenBank genome annotations.

We characterized mitochondrial genomes based on gene order and genome size, where genomes with gene arrangements that deviated from the consensus vertebrate mitochondrial gene order and genomes that were expanded (> 17.5 kb) were labeled as “modified” (in contrast to “normal”). Gene order was described relative to the control region (the initiation site for mtDNA replication and transcription), beginning with tRNA-Phe, using a purpose-built perl script (Minxiao et al. 2011). We extracted mitochondrial genome size data from the GenBank files. These results are summarized in Table A2.1, Table A2.2, and Figure A2.1.

Compositional analyses were carried out for all genomes in the dataset using custom perl scripts (available by request from author). For each of the 13 protein-coding genes, we estimated base composition for the whole gene and for the third position of four-fold degenerate codons (P_{4FD}): alanine-GCN, glycine-GGN, leucine-CTN, proline-CCN, arginine-CGN, serine-TCN, threonine-CAN, and valine-GTN. Base frequencies, as well as AT skew and GC skew, were also calculated for the whole genome. These results are summarized in Table A2.1. We tested for differences in AT skew and GC skew values between normal and modified genomes using a two-way Mann-Whitney Test implemented in R.

Initial phylogenetic analysis

Multiple sequence alignments were performed based on amino acid sequences for each mitochondrial protein-coding gene using MUSCLE v.3.8 (Edgar 2004). We then estimated a maximum likelihood tree for the concatenated mitochondrial gene dataset using RAxML v.7.2 (Stamatakis 2006). The data were partitioned by gene and codon position and analyzed using the GTR + Γ model of nucleotide substitution for each partition.

Nonsynonymous and synonymous substitution analysis

For each mitochondrial gene, we estimated nonsynonymous (dN) and synonymous (dS) substitution rates. We first estimated dN and dS using a single value for each parameter across all branches (Model 0 in Codeml, implemented in PAML v4.4 (Yang 2007)) using the fixed topology of the maximum likelihood tree estimated from the concatenated mitochondrial sequences. Subsequently, we re-estimated these parameters under Model 1 (in Codeml), which allows these parameter values to vary for each branch. These results are summarized in Table A2.2. For each gene, we tested for differences in dN and dS values between normal and modified genomes using a two-way Mann-Whitney Test implemented in R.

Mutation gradient analysis

The site-specific mutation rate around the mitochondrial genome is associated with the duration of time spent single stranded, which is determined by the position of a site relative to the origins of replication. The duration of single-stranded state of the parental H strand (D_{ssH}) is defined by the duration between the displacement of the heavy strand by the replication fork and synthesis of its complement; for a given gene, it is estimated using the formula $D_{ssH} = (L - 2(x - O_L)) / L$ for the two genes located upstream of the origins of replication, ND1 and ND2, and $D_{ssH} = (2(x - O_L)) / L$ for the remaining genes, where L is the total length of the genome, O_L is the position of the light strand origin of replication, and x is the midpoint of a gene (Tanaka and Ozawa 1994; Reyes et al. 1998). We used a modification of these formulas to estimate absolute mutation position, which is not standardized by genome size, for each mitochondrial gene for all genomes because our goals include comparing estimates of mutation position for a given gene across genomes of different sizes. Specifically, we hypothesize that rearrangements and

expansions may alter the mutation position of genes in modified genomes. Mutation position is estimated using $(L - 2(x - O_L))$ for the two genes located upstream of the origins of replication, ND1 and ND2, and $(2(x - O_L))$ for the remaining genes.

Functional constraint on mitochondrial genes

To test whether mitochondrial genome expansions and rearrangements reflect an overall relaxation of selective constraint on mitochondrial function, we estimated ω , which is the ratio of nonsynonymous to synonymous substitutions ($\omega = dN/dS$), for each mitochondrial gene. The ratio ω is used to measure the strength of selection, where for values of ω between zero and one, a smaller ω indicates stronger purifying selection and a larger ω indicates weaker purifying selection. We first estimated ω using a single value across all branches (Model 0 in Codeml, implemented in PAML v4.4 (Yang 2007)) using the fixed topology of the concatenated mitochondrial maximum likelihood tree. We then re-estimated ω under Model 1 where parameter values can vary for all branches. These results are summarized in Table A2.2. For each gene, we tested whether ω was greater in modified genomes using a one-way Mann-Whitney Test implemented in R.

RESULTS AND DISCUSSION

Mitochondrial genome characteristics

For mitochondrial genomes from 62 salamander species, we estimated genome composition and organization to identify and characterize the differences between normal and modified genomes. We identified 14 mitochondrial genomes as modified based on evidence of gene rearrangement, a large increase in genome size (>17.5 kb total length), or a combination of

the two. Eight of these modified genomes show extensive gene rearrangements that include one or more of the origins of replication and the regions flanking them, including both protein-coding genes and tRNAs. The remaining six modified genomes maintain the ancestral vertebrate gene order, but are larger than 19 kb in size. This size increase reflects the accumulation of tandem repeats of non-coding sequence in the control region and/or in the IGS, an intergenic spacer region between tRNA-Thr and tRNA-Pro present in diverse salamander clades (Wallis 1987; McKnight and Shaffer 1997; Mueller and Boore 2005). Similar patterns of non-coding sequence accumulation exist in other taxonomic groups (e.g. mammals, caecilians, fish, invertebrates) (Stewart and Baker 1994; Prager et al. 1996; Delarbre et al. 2001; San Mauro et al. 2006; Minxiao et al. 2011). Tandem repetitive non-coding sequences are also present in seven of the eight genomes with rearranged gene order. Overall, genome size is significantly larger in modified salamander genomes ($19,775 \pm 1809$ bp) than in normal salamander genomes (16,475 ± 209 bp). Both pseudogenes and additional copies of duplicate genes are present in at least six of the modified salamander mitochondrial genomes, suggesting that these rearrangements were mediated by duplication of a portion of the genome (Mueller and Boore 2005). The localization of genomic modification to regions containing and flanking the two origins of replication, as well as the presence of tandem repetitive sequences, suggests that these genomic regions are particularly susceptible to slipped-strand mispairing, intramolecular recombination, and imprecise replication.

We estimated several measures of genome composition for normal and modified genomes and compared the two groups. Average base composition did not differ between normal (A: 0.3352, T: 0.3104, C: 0.1390, G: 0.2152) and modified genomes (A: 0.3414, T: 0.3029, C: 0.1307, G: 0.2250). Average GC content also did not differ between the two groups (35.4% and

35.5%, respectively). In contrast, normal and modified genomes did differ slightly in both average AT skew (0.039 and 0.046, respectively; $p = 0.087$) and GC skew (-0.214 and -0.226, respectively; $p < 0.001$).

Substitution rates in normal and modified genomes

To test for differences in rates of evolution between normal and modified genomes, we estimated rates of synonymous (dS) and nonsynonymous (dN) substitution across a maximum likelihood tree for each of the 13 mitochondrial protein-coding genes. Our results show that mitochondrial genes in modified genomes have a significantly elevated dS ($p < 0.001$ for all genes) and dN ($p < 0.006$ for all genes). Modified genomes show, on average, a 2.66-fold increase in dS compared to normal genomes; COX1 shows the smallest increase (2.31-fold), while ATP6 shows the greatest increase (3.17-fold). Modified genomes show, on average, a 3.07-fold increase in dN compared to normal genomes; ND4 shows the smallest increase (2.42-fold) and COX2 shows the largest increase (4.50-fold). These results demonstrate that mitochondrial genome modification is correlated with an absolute increase in both synonymous and nonsynonymous substitution rates for mitochondrial protein-coding genes, independent of phylogeny (Figure 2.2).

Although the mechanisms that give rise to the correlation between substitution rate and mitochondrial rearrangement remain unknown in salamanders as well as other taxa, a number of hypotheses have been proposed. Some such hypotheses state that an increase in substitution rates causes elevated rates of gene rearrangement. Specifically, increased substitutions accumulating in the sequences regulating replication initiation and termination can decrease replication fidelity, resulting in duplications, deletions, and rearrangements (Shao et al. 2003). The high

rates of substitution may also cause high rates of DNA damage and double-strand breaks, which can facilitate intramolecular recombination (Downton and Campbell 2001). Alternatively, the presence of gene rearrangements may cause an increase in substitution rates by an undetermined mechanism. Differences in population biology have also been proposed to explain the correlation between elevated substitution rates and genomic modifications; relatively strong genetic drift would cause fixation of both point mutations and large-scale genomic modifications (Lynch et al. 2006; Boussau et al. 2011). Comparative studies that test for the signatures of these putative molecular and demographic mechanisms are required to determine the cause of this correlation in salamanders as well as other taxa.

Mutation gradient in normal and modified genomes

To test for differences in gene placement along the mutation gradient between normal and modified mitochondrial genomes, we first estimated the average mutation position for each gene in each species. We define average mutation position as the duration spent single-stranded, measured in base pairs. This measurement reflects the probability of mutation for a given position in the mitochondrial genome due to its exposure, in the single-stranded state, to the mutagenic mitochondrial environment during replication. We do not standardize for total genome size, as done in previous studies on mitochondrial mutational gradients (Tanaka and Ozawa 1994; Faith and Pollock 2003; Krishnan et al. 2004), because our goals include comparing estimates of mutation position for a given gene across genomes of different sizes. Specifically, we hypothesize that rearrangements and expansions may alter the mutation position of genes in modified genomes. Our results show that alterations to mutation position are restricted to a subset of genes in modified genomes. Specifically, genes located upstream of both

the O_L and the O_H — ND1 and ND2 (Figure 2.1) — experience a large increase in average mutation position in modified genomes (3.3 ± 1.9 kb each) (Figure 2.3). This pattern reflects the fact that the majority of genomic expansions occur near the O_H . During replication, such expansions are not encountered until replication has begun from the O_L (Figure 2.1) and completed the majority of both strands. Therefore, only genes located upstream of both the O_L and the O_H — ND1 and ND2 — spend a longer duration in the single-stranded state in expanded genomes. Of the remaining 11 genes positioned downstream of both replication origins, nine are never involved in gene rearrangements and have very similar mutation positions in both modified and normal genomes; on average, these genes shift less than 70 bps (Figure 2.3). In contrast, ND6 and CYTB have been involved in four independent gene rearrangements (Mueller and Boore 2005). Mutation position of ND6 either decreases by 4.0 kb or increases by up to 8.5 kb, depending on lineage-specific gene rearrangements. Mutation position of CYTB either decreases by 1.2 kb or increases by up to 2.5 kb, depending on lineage-specific gene rearrangements.

We then tested for a gradient generated by mutational bias during replication in normal and modified salamander genomes. We estimated base compositional asymmetry (i.e. AT skew) of the third position of four-fold degenerate codons (P_{4FD}) for each gene. A linear regression of AT skew on mutation position indicates significant positive correlation for normal genomes, though only a small proportion of compositional variation can be explained by mutation position ($y = 0.000006874x + 0.271$, $r^2 = 0.056$, $p < 0.001$). The positive relationship between AT skew and mutation position is not significant in modified genomes ($y = 0.000003913x + 0.318$, $r^2 = 0.018$, $p = 0.068$), suggesting that modified genomes are not at the same base compositional equilibrium as normal salamander genomes. However, this mutational gradient does not translate

into a gradient in either dN or dS (Figure 2.3). Thus, although the mutation positions of ND1, ND2, ND6, and CYTB are altered during genomic expansion and/or rearrangement, their movement to this new position has no significant impact on their substitution rates. Salamanders have the lowest aerobic metabolic demands of any tetrapod vertebrates, suggesting that their levels of reactive oxygen species (ROS, a mutagenic byproduct of oxidative phosphorylation) may be low. This may underlie their weak mitochondrial mutational gradient, although comparative studies of this gradient across taxa with different metabolic rates are required to test this hypothesis.

Functional constraints on mitochondrial genes in normal and modified genomes

To test whether mitochondrial genome expansions and rearrangements reflect an overall relaxation of selective constraint on mitochondrial function, we compared estimates of ω (dN/dS , a measure of the strength of selection) for all 13 mitochondrial genes between normal and modified genomes. Specifically, we tested whether ω is greater in modified genomes, indicative of weaker selective constraint. Although both dN and dS are elevated for all genes in modified genomes, ω values for genes in modified genomes are not significantly greater than ω values in normal genomes (Figure 2.4; $p > 0.189$ for all genes). Thus, genes within modified genomes are not experiencing weaker selective constraint than genes within normal genomes. This result demonstrates that mitochondrial genome expansions and rearrangements likely do not reflect an overall relaxation of selective constraint on mitochondrial function. Such an overall relaxation would affect protein sequence evolution as well as transcriptional and translational efficiency, which are impacted by changes in genome size and gene order (Fernandez-Silva et al. 2003; Bonawitz et al. 2006; Satoh et al. 2010; Chong and Mueller 2013). In contrast, our results show

that functional constraints on protein-coding sequences are not weaker in lineages with modified genomes than in lineages with normal genomes.

FIGURES

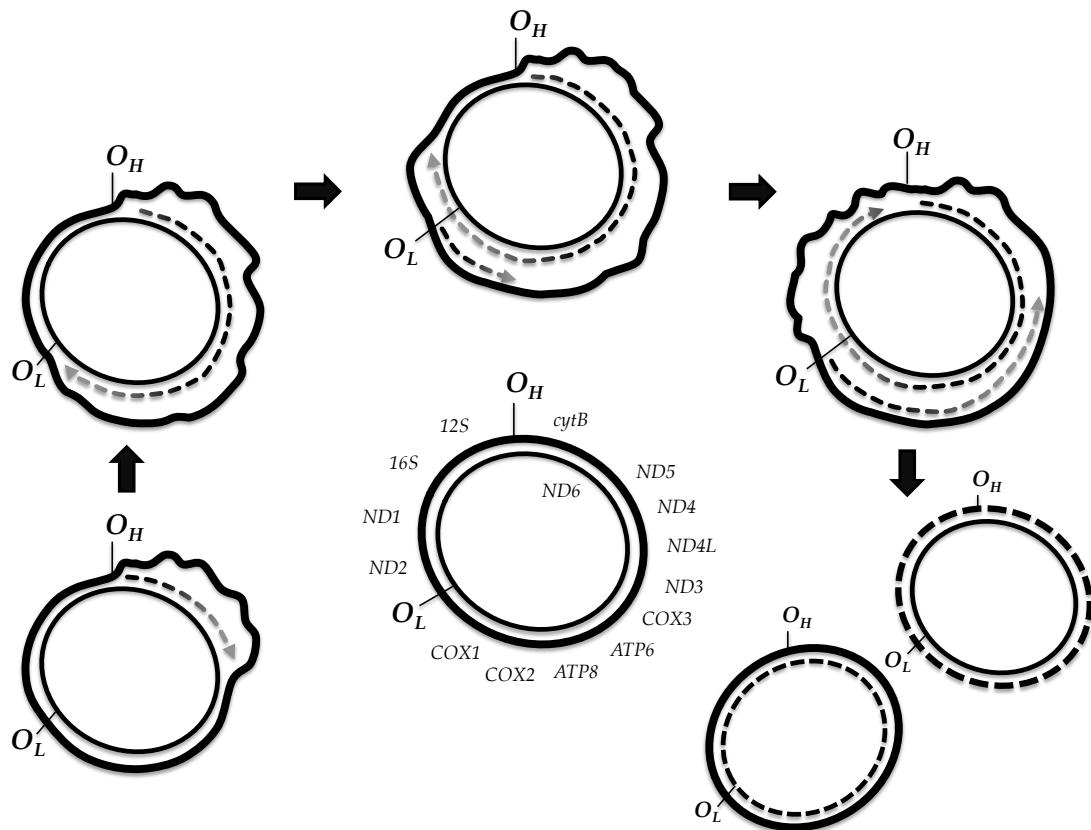


Figure 2.1 Schematic representation of vertebrate mitochondrial genome replication. Replication begins at the O_H and proceeds along the heavy strand. Once the replication fork passes the O_L , replication begins along the light strand.

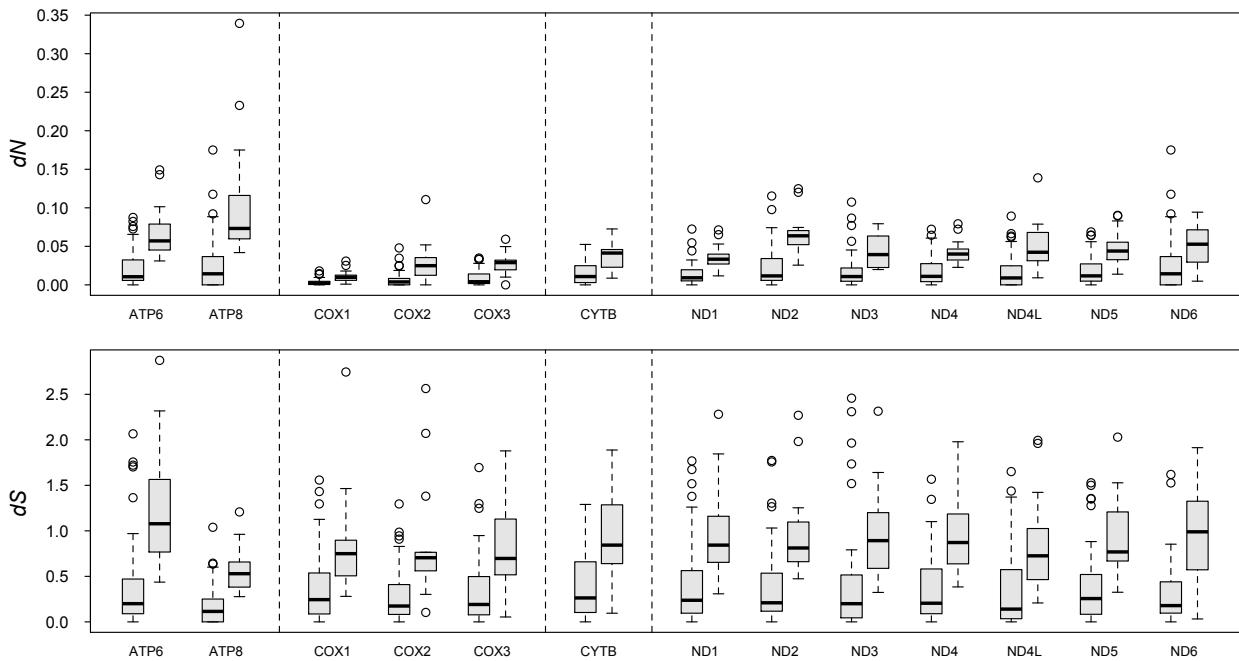


Figure 2.2 Comparison of nonsynonymous and synonymous substitution rates for the 13 mitochondrial protein-coding genes. Normal genomes are on the left; modified genomes are on the right. The dashed lines separate mitochondrial genes that belong to different OXPHOS function complexes. For all comparisons, substitution rates of modified genomes are significantly higher than substitution rates of normal genomes.

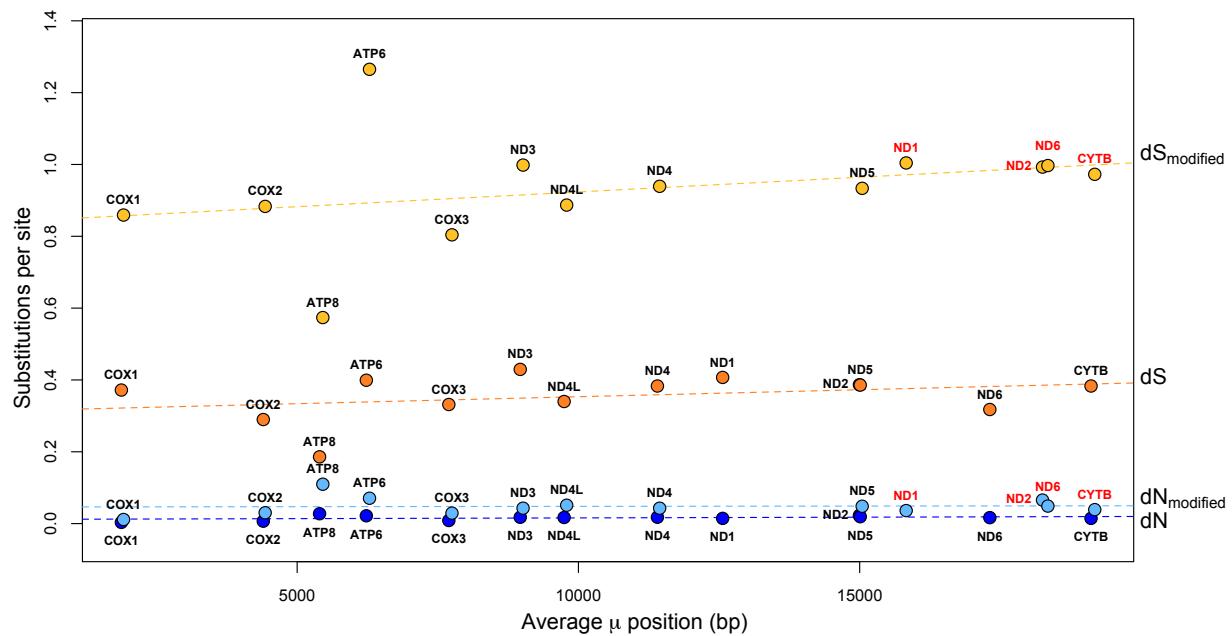


Figure 2.3 Plot of average mutation position (x-axis) and average nonsynonymous (dN) and synonymous (dS) substitution rates (y-axis) for each mitochondrial gene for both normal and modified genomes. Average dN for normal mitochondrial genomes are dark blue, average dN for modified mitochondrial genomes are light blue, average dS for normal mitochondrial genomes are dark orange, and average dS for modified mitochondrial genomes are light orange. The mutation position for a given gene is the average time spent single stranded measured in base pairs. Genes within modified genomes do not significantly differ in mutation position from genes within normal genomes, with the exception of four genes (labeled red): average mutation positions of ND1 and ND2 increase by 3.3 kb within modified genomes. Mutation position of ND6 either decreases by 4.0 kb or increases by up to 8.5 kb within rearranged genomes, depending on lineage-specific gene rearrangements. Mutation position of CYTB either decreases by 1.2 kb or increases by up to 2.5 kb, depending on lineage-specific gene rearrangements. Despite the presence of a weak mutational gradient, no significant relationship exists between average mutation position and substitution rates (dN or dS) in salamanders. Thus, change in mutation position has no significant impact on rates of molecular evolution.

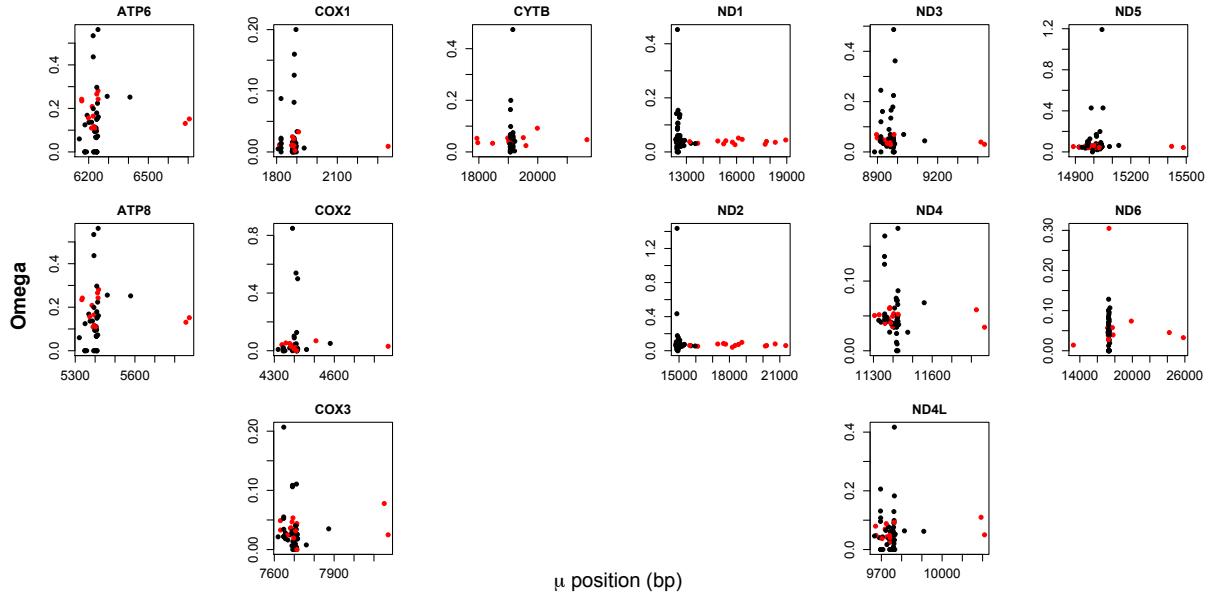


Figure 2.4 Plots of levels of selective constraint, represented by ω (dN/dS), acting on the 13 mitochondrial genes for both normal (black) and modified (red) genomes. Omega plots for each gene are grouped to reflect protein functional complexes (i.e. COX1-3, ATP6&8, CYTB, ND1-6 and 4L). ω values for modified genomes are not larger than those for normal genomes, showing that genes in modified genomes are not experiencing weaker selective constraint.

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CHAPTER 3

DUPLICATION AND NOT-SO-RANDOM LOSS IN

REARRANGED MITOCHONDRIAL GENOMES OF SALAMANDERS

INTRODUCTION

Mitochondria are the site for the citric acid cycle and oxidative phosphorylation (OXPHOS), the final steps of ATP synthesis via cellular respiration. These intracellular organelles have their own genome, which in vertebrates is a small, circular molecule averaging 16.5 kb in length (Boore 1999; Scheffler 2008). These small genomes encode 13 electron transport proteins, two rRNAs, and 22 tRNAs (Scheffler 2008). Although they have evolutionarily conserved genomic content, metazoan mitochondrial genomes show a diversity of gene orders in some taxa (Boore and Brown 1998; Boore 1999; Saccone, et al. 1999; Xu, et al. 2006). Mitochondrial gene rearrangements can span both large and small portions of the genome, and different gene orders have been observed in divergent taxa.

Several mechanisms have been proposed to explain mitochondrial gene rearrangement. In the duplication-random loss (DRL), a gene or sequence of genes is duplicated in tandem; subsequently, one copy sustains a loss of function mutation, thereby fixing the alternate copy (Moritz, et al. 1987; Boore 2000). This model has been invoked to explain the variation in mitochondrial gene order observed among whiptail lizards, geckos, insects, caecilians, and other animals (Mortiz and Brown 1987; Shao, et al. 2003; San Mauro, et al. 2006; Xu, et al. 2006; Fujita, et al. 2007). Although patterns of gene order variation and the presence of pseudogenes in the genomes of some taxa suggest that DRL occurs, little is known about how random loss actually occurs. For example, duplicate gene copies (initially intact, but decaying to

pseudogenes) will be present in the mitochondrial genome for some period of time, but it remains unclear for how long. The presence of duplicate gene copies may negatively impact mitochondrial function by increasing the time required to complete (1) transcription and translation of mitochondrial genes, and/or (2) replication of the mitochondrial genome. Additionally, transcription and translation of mitochondrial pseudogenes may result in the production of non-functional proteins, which could negatively impact mitochondrial function (Schuster and Brennicke 1991). Finally, transcription and translation of additional functional gene copies may result in changes to the stoichiometry of mitochondrial proteins (Rossi and Lehninger 1964; Papa and Skulachev 1997), which could negatively impact mitochondrial function. If the presence of duplicate gene copies is deleterious for any of these reasons, then deletion events should be advantageous; selection should drive rapid excision from the genome. Alternatively, if the presence of duplicate gene copies is selectively neutral, then deletions will be fixed by genetic drift, leading to relatively slow loss of duplicate sequences and their persistence in the genome for prolonged periods of time.

Different mitochondrial gene orders have been documented across highly divergent clades such as bird families and amphibian orders (McKnight and Shaffer 1997; Boore 1999; Haring, et al. 2001; Mueller and Boore 2005). Our current understanding of gene order variation primarily arises from comparisons of such taxa, where rearrangements have been fixed deep in evolutionary time. In these systems, the intermediate stages of random loss have occurred far enough in the past that little or no trace of excess duplicated sequence remains in the genomes of extant taxa. Comparisons of gene order variation among more closely related taxa, where the intermediate stages of random loss remain in the genome, remain relatively rare. By analyzing mitochondrial genomes from closely related species where a lineage has recently experienced a

duplication event, we can understand the process of random sequence loss, which would shed light on the dynamics of how mitochondrial gene rearrangements arise.

Salamanders, an amphibian clade of 663 species, include both “normal” vertebrate mitochondrial genomes and a range of independently derived, modified genome architectures that include gene rearrangements. Because this mitochondrial genomic diversity is rare among vertebrates, salamanders provide an unusual opportunity to understand how mitochondrial gene rearrangements arise in vertebrate genomes. The North American salamander genus *Aneides* is comprised of six species that include two divergent lineages (*A. hardii* and *A. flavipunctatus*) with distinct mitochondrial gene orders (Mueller, et al. 2004; Mueller and Boore 2005), both of which differ from the normal vertebrate gene order. Specifically, the mitochondrial gene order observed in *A. flavipunctatus* is hypothesized to be the result of a duplication spanning protein coding genes ND6, tRNA-E, cytB, tRNA-T, the IGS (an intergenic spacer between tRNA-T and tRNA-P that is found in diverse salamander clades (McKnight and Shaffer 1997; Zhang, Chen, Liu, et al. 2003; Zhang, Chen, Zhou, et al. 2003)), and tRNA-P; these duplicate copies subsequently decayed to pseudogenes and resulted in the complete excision of one copy of ND6 and tRNA-E and the extreme decay of one copy of cytB and tRNA-T (Mueller and Boore 2005). The mitochondrial genome of *A. hardii* shows evidence of having undergone two duplication events. The first duplication involves a nearly identical region of the genome observed in *A. flavipunctatus*, which suggests that this first duplication event is either a synapomorphy or convergent between these two species. The second duplication in *A. hardii* is hypothesized to include part of the first duplication as well as the control region, tRNA-F, 12S, tRNA-V, 16S, tRNA-L, and ND1 (Mueller and Boore 2005).

For this study, we examined mitochondrial gene order in individuals from multiple population-level lineages of *Aneides* (both within and among species) in a phylogenetic context to pinpoint the evolutionary origins of gene duplications in the clade. Our goals were (1) to determine whether variation in gene order exists between any closely related lineages, and (2) use any such lineages to examine the process of pseudogene loss. In this system, we find evidence for two separate duplication events that result in mitochondrial gene rearrangements. Our results show that mitochondrial gene order can vary significantly between closely related populations of the same species. We also demonstrate that after a duplication event, protein coding and rRNA genes decay more quickly relative to other duplicated sequences, and the decay of duplicate protein-coding genes and near complete removal of duplicate rRNA genes occurs over very shallow levels of genetic divergence. Thus, we conclude that mitochondrial genome duplication events are followed by rapid and nonrandom loss of sequence, suggesting that selection is acting to quickly remove these duplicate gene copies.

METHODS

Taxon selection

Our first goal was to construct a comprehensive phylogeny for the clade in order to analyze mitochondrial gene rearrangements in an evolutionary context. Therefore, we collected nuclear sequence data from at least four individuals for all six species of *Aneides* (Table 3.1). In an attempt to maximize representation of population-level lineages, we included additional samples of *A. flavipunctatus* because previous studies have documented high levels of divergence across different populations (Pope and Highton 1980; Rissler and Apodaca 2007). We also included four closely related species as outgroups (*Ensatina eschscholtzii*, *Hydromantes*

brunus, *Plethodon elongatus*, and *Desmognathus fuscus*) (Mueller, et al. 2004; Vieites, et al. 2007; Vieites, et al. 2011) for our phylogenetic analyses. In order to analyze gene order to pinpoint the evolutionary origins of these duplications in the clade, we sampled genomes from individuals that represent nine divergent populations-level lineages of *Aneides*, both within and among species (Table 3.2). Specifically, we sampled the same individuals for both datasets to maximize overlap between the two datasets where possible. For within species comparisons, we sampled multiple *A. hardii* and *A. flavipunctatus* individuals.

DNA amplification and sequencing for phylogenetic analyses

For each sample, we extracted total genomic DNA from either flash-frozen or RNAlater (Qiagen) preserved liver or tail tissue using Qiagen Puregene kit protocols. We amplified a portion of the nuclear encoded genes BDNF, POMC, and RAG1 using Polymerase Chain Reaction (PCR), which produced amplicons of 720 bps, 489 bps, and 837 bps for each gene, respectively. Primers and PCR reaction conditions were obtained from previously published studies (Vieites, et al. 2007). Briefly, approximately 10 ng of genomic DNA was amplified using 0.25 mM dNTPs, 0.5uM of each primer, 0.5 U *Taq*, 25uM MgCl₂ and 10x reaction buffer (no MgCl₂) in a 12.5uL reaction volume. Genes were PCR amplified for 35 cycles (95°C 45s, 56-58°C 1min, and 72°C 1min). All PCR products were electrophoresed on a 1% agarose SB gel stained with Gel Red (Biotium Inc.) and visualized under ultraviolet light. PCR products were purified using ExoSAP-IT (USB Corp.). PCR products were sequenced in both directions by the University of Chicago Comprehensive Cancer Center's DNA sequencing and genotyping facility using direct double strand cycle sequencing with Big Dye v3.1 chemistry (Perkin-Elmer) and an ABI 3730 automated sequencer. Contiguous DNA sequences were aligned and edited using

Geneious version R7 (created by Biomatters), and multiple sequence alignments were initially generated using Muscle v.3.6 (Edgar 2004) and subsequently verified by eye. The open reading frames were also verified for all genes using Geneious R7.

Nuclear gene phylogenetic analyses

We estimated phylogenetic relationships using both Maximum Likelihood (ML) and Bayesian Inference (BI) on the nuclear dataset (BDNF, POMC, and RAG1) to infer the evolutionary history of the genus *Aneides*. We evaluated multiple data partitioning strategies in order to incorporate evolutionary information specific to each gene and codon position. We determined three appropriate data partitions using PartitionFinder v.1.1.1 (Lanfear, et al. 2012), which include (1) codon positions 1 and 2 for BDNF, (2) codon positions 1 and 2 for both POMC and RAG1, and (3) codon position 3 for all three nuclear genes. Using Akaike Information Criterion to select the best model of nucleotide substitution, we determined JC, F81 + I, and GTR + G for each partition, respectively.

Partitioned Bayesian analyses were conducted in MrBayes v.3.2 (Ronquist, et al. 2012) for the concatenated nuclear dataset. We conducted two independent searches consisting of three “heated” and one “cold” Markov chain for 10 million generations with every 1000th sample retained. A rate multiplier was used to allow substitution rates to vary among partitions and default priors were applied to all model parameters. We assessed convergence of the MCMC using several diagnostics. We viewed trace plots of tree -lnL values and other parameters in Tracer v.1.5 (Rambaut and Drummond 2007). Trees sampled prior to stationarity were considered as burn in and resulted in the first 1000 tree samples being discarded. To determine whether the two independent runs converged on similar results, we examined the split standard

deviation for -lnL tree values among chains; values <0.01 indicate convergence. We also used the program Are We There Yet? [AWTY (Nylander, et al. 2008)] to compare changes in the posterior probabilities of split frequencies across the independent runs.

Partitioned Maximum Likelihood analyses were conducted using RAxML v.7.2.5 (Stamatakis 2006) under the GTR + Γ model of nucleotide substitution for all data partitions identified in the Bayesian analysis. Support values for the inferred relationships were obtained from 1000 nonparametric bootstrap pseudoreplicates.

Mitochondrial genome sequencing and assembly

We obtained cellular DNA shotgun sequence data, which includes both nuclear and mitochondrial DNA, using the Illumina MiSeq sequencing platform for a total of eight individuals representing all six species of *Aneides*, with additional sampling within *A. hardii* and *A. flavipunctatus* (Table 3.2). Illumina sequencing libraries were produced for each sample using the IntegenX PrepX-DNA 24 library prep kit (IntegenX). Prepared libraries were pooled equimolar for sequencing, allocating $\frac{1}{3}$ of a 2x250 cycle MiSeq run (Illumina) for all samples. Library preparation and sequencing were performed by the University of Idaho Institute for Bioinformatics and Evolutionary Studies (IBEST) Genomics Resources Core facility. We screened shotgun reads from each of our focal taxa to eliminate sequencing adapters, identify and remove contaminants, and trim reads based on quality scores using the bioinformatics pipeline “SeqyClean.py” provided by the IBEST Computational Resources Core. We assembled the remaining shotgun reads into contigs using Newbler v.2.6. We also included published 454-whole-genome shotgun sequence data for an additional sample of *A. flavipunctatus* (Sun, Shepard, et al. 2012) for a total of nine individuals.

For each of the nine shotgun sequence datasets, we identified contigs of mitochondrial sequences using tBLASTx, where contigs were used as queries to BLAST against mitochondrial genome reference sequences of *A. hardii* (AY728226) and *A. flavipunctatus* (AY728214), with an e-value cutoff of 1e-5. Whole or nearly complete mitochondrial genomes were represented by one to eight contigs. Several genome assemblies included gaps across regions of low sequencing coverage. For these regions, we developed genome-specific primers for PCR amplification and Sanger sequencing to minimize sequence gaps and to verify the assemblies of multiple contigs.

Mitochondrial gene order identification

We annotated mitochondrial genome sequences for each individual using Geneious R7. rRNAs and tRNAs were identified based on sequence similarity with published genomes. All 13 protein-coding sequences were verified by eye for appropriate vertebrate mitochondrial open reading frames and stop codons. For each sample, we determined mitochondrial gene order based on our genome annotations and assembly to reference mitochondrial genomes. We also searched for the presence of pseudogenes based on levels of sequence similarity to the functional copy and positions relative to flanking sequences.

Pseudogene decay analysis

For closely related lineages of *A. hardii* where a duplication was present in one lineage, but not in its sister taxon, we examined the extent of pseudogene decay relative to substitution accumulation. We scaled pseudogene loss by substitutions because the limited salamander fossil record prevents us from accurately estimating a time-calibrated phylogeny necessary for measuring the absolute rate of pseudogene decay (Sun, Lopez Arriaza, et al. 2012). More

specifically, we estimated the evolutionary genetic distance between two populations of *hardii* (two individuals for each population). We generated multiple sequence alignments for each of the protein-coding genes using translational ClustalW alignment (Larkin, et al. 2007) and subsequently verified alignments by eye for open reading frames and stop codons. We then calculated the average number of nucleotide substitutions per site between populations (D_{xy}) for a concatenated dataset of all 13 protein-coding genes for each sample using DnaSP v 5.10.1 (Librado and Rozas 2009). We used Jukes and Cantor's model of nucleotide substitution, which is likely sufficient for such shallow divergences.

RESULTS AND DISCUSSION

Aneides phylogeny estimated from nuclear genes

Despite recent work on salamander phylogenies (Kalinowski, et al. 2007; Vieites, et al. 2007; Pyron and Wiens 2011; Vieites, et al. 2011), the phylogenetic relationships within *Aneides* were previously unknown. From our analysis of 2,046 bp from three nuclear genes (BDNF, POMC, and RAG1), we inferred a strongly supported phylogeny for the genus that included all six species represented by several divergent lineages, thus, providing the most comprehensive multi-locus phylogeny of the group to date (Figure 3.1). Specifically, our results show *A. aeneus* to be sister to the rest of the clade; *A. aeneus* is the only species found in eastern North America, while the remaining five species are only found in western North America. The two Pacific Northwest species *A. ferreus* and *A. vagrans* are sister taxa, which together form the clade sister to *A. flavipunctatus*. These three species (*A. flavipunctatus*, *A. ferreus*, and *A. vagrans*) are sister to *A. lugubris*, and these four collectively form the clade found along the coastal regions of Western North America. *Aneides hardii*, which can only be found in a few isolated mountaintops

in southern New Mexico, is sister to the west coast North American clade. Our results are congruent with the relationships suggested by other phylogenetic studies that have included some of the species of *Aneides* (Mueller, et al. 2004; Vieites, et al. 2007; Pyron and Wiens 2011; Vieites, et al. 2011).

Mitochondrial gene order

We identified mitochondrial gene orders for nine *Aneides* samples using shotgun sequence data. The total amount of sequence and average read length varied among samples, but on average it represents approximately 0.5-1.5% of the nuclear genome at 1x coverage (Table 3.2). We identified nearly complete mitochondrial genome sequences from a single contig for two samples. For each of the remaining samples, we were able to identify four to eight contigs that represented large fractions of the mitochondrial genome. We eliminated several assembly gaps using PCR; however, several regions of the genome still remain elusive. Specifically, the region between CYTB and ND6, as well as the control region, was very difficult to obtain for nearly all samples, which may be a result of these two regions being composed of highly repetitive sequences of varying length, making it very difficult to assemble. In the end, we were able to sequence and annotate nearly complete mitochondrial genome sequences for all nine samples. We combined these data with two previously published genomes (one *A. hardii* and one *A. flavipunctatus* individual) for a total of 11 *Aneides* mitochondrial genome sequences (Table 3.2).

We found evidence of gene rearrangement, relative to the typical vertebrate mitochondrial gene order, in all 11 mitochondrial genome sequences. Specifically, the mitochondrial gene order in *A. aeneus*, *A. lugubris*, *A. vagrans*, *A. ferreus*, and all sampled

lineages of *A. flaviguttatus* shows a pattern of gene order exchange between ND6 and CYTB as well as the accumulation of DNA in this region (Figure 3.2A). Based on these results, we can infer that this gene rearrangement is a synapomorphy for *Aneides*, which suggests that the duplication event and gene rearrangement occurred at the base of the clade (Figure 3.1).

Pseudogene decay

Of the three *A. hardii* mitochondrial genomes we analyzed, only one had the novel gene order (reflecting two duplication events) previously observed in this species (hereafter Ahar2) (Mueller and Boore 2005). The other two had the gene order we show here to be ancestral for *Aneides* (hereafter Ahar1). Thus, we show two different mitochondrial gene orders within the single species of *A. hardii* (Figure 3.2). Capturing this relatively recent duplication event within *A. hardii* gave us a rare opportunity to examine random sequence loss leading to gene rearrangement in some detail.

The average number of nucleotide substitutions per site between populations of *A. hardii* with different gene orders (i.e. between Ahar1 and Ahar2) was low ($D_{XY} = 0.024$). This result is congruent with our nuclear gene phylogenetic analysis, which shows very low genetic diversity within *A. hardii* (Figure 3.1). Thus, all of the gene loss we identify in Ahar2 has taken place in the time taken to accumulate only 2.4% sequence divergence from Ahar1 mitochondrial genomes. We note that, because we sampled only two individuals per *A. hardii* population, we are likely falsely identifying some sites as fixed differences between the two populations, which would produce inflated estimates of D_{XY} ; thus, the two populations of *A. hardii* may be even less genetically differentiated than we report here.

Since diverging from Ahar1 mitochondrial genomes after the duplication event, Ahar2 genomes have lost several duplicated genes and accumulated noncoding DNA. Overall, the duplicated protein coding genes and ribosomal genes are lost more rapidly than other duplicated sequences (e.g. tRNAs, control region, IGS). 12S, tRNA-Val, 16S, ND1, and one copy of ND6 have all been partially or completely removed from the genome. Copies of both rRNAs and the intervening tRNA-Val, the functional copies of which total 2,520 bp, have been reduced to a 111 bp segment of unrecognizable sequence. The pseudogene copy of ND6 is 621 bp with a sequence similarity of 97% to the functional copy, the pseudogene copy of ND6 differs from the function copy by ten point substitutions and two insertions, which result in several amino acid replacement substitutions to stop codons. The pseudogene copy of ND1 is a 670 bp fragment that is 92% similar to the first region of the functional ND1; this ψ ND1 may be the result of the Ahar2 duplication spanning ND1 only partially, which would produce an immediate pseudogene because of its shortened length. In contrast to the decay and removal of protein coding genes and rRNAs, all duplicated tRNAs (except tRNA-Val, located between the two rRNAs) likely remain functional; both the stem-loop structures and the anticodon sequences are intact. Additionally, the sections of noncoding sequence from the ancestral *Aneides* duplication that are predicted to be the remnants of a decayed copy of CYTB + tRNA-Thr + IGS and a decayed copy of IGS + tRNA-Pro are retained in duplicate copy. Based on our results, we infer that after the *hardii*-specific duplication, Ahar2 genomes have both (1) lost function of, and/or completely removed, all duplicate protein coding and rRNA genes, (2) retained most duplicate tRNAs, and (3) retained and/or continued to accumulate noncoding DNA.

Our results suggest that after duplication events, extra mitochondrial gene copies decay rapidly relative to other duplicate sequences. This pattern is consistent with selection playing a

role in the removal of these duplicate proteins, either before or after mutation alters their sequences. Selection could act to maintain stoichiometry among functional protein products immediately following duplication, when gene duplicates are still identical in sequence. In addition, selection could act to eliminate non-functional proteins from the proteome after gene duplicates have sustained loss-of-function mutations. We note that, in the case of rRNAs, additional functional copies would not be expected to be removed by selection.

If genome size itself were under selection, because of its effects on time required to complete (1) transcription and translation of mitochondrial genes, and/or (2) replication of the mitochondrial genome, then we would predict that the removal of all duplicate sequences would be advantageous. However, the persistence of some duplicate DNA sequences — tRNAs, regions consisting of repetitive noncoding sequence — suggests that these sequences may be selectively neutral whereas duplicate genes may be disadvantageous. Based on our results, we suggest that the process of duplication and random loss may be less random than previously predicted.

TABLES

Table 3.1 Specimen information for individuals included in this study and their voucher numbers and locality information

Species	Voucher	State: County	Source
<i>Aneides aeneus</i>	DH74978	GA: Chatooga	<i>This study</i>
<i>Aneides aeneus</i>	DH74985	GA: Chatooga	<i>This study</i>
<i>Aneides aeneus</i>	DH77583	KY: Letcher	<i>This study</i>
<i>Aneides aeneus</i>	DH77584	KY: Letcher	<i>This study</i>
<i>Aneides ferreus</i>	MVZ219942	CA: Siskiyou	<i>This study</i>
<i>Aneides ferreus</i>	MVZ219953	OR: Douglas	<i>This study</i>
<i>Aneides ferreus</i>	MVZ219958	OR: Linn	<i>This study</i>
<i>Aneides ferreus</i>	RCT545	CA: Del Norte	<i>This study</i>
<i>Aneides flavipunctatus</i>	AGC299	CA: Santa Cruz	<i>This study</i>
<i>Aneides flavipunctatus</i>	MVZ219973	CA: Siskiyou	Vieites et al. 2007
<i>Aneides flavipunctatus</i>	MVZ219977	CA: Sonoma	<i>This study</i>
<i>Aneides flavipunctatus</i>	RAC080	CA: Mendocino	<i>This study</i>
<i>Aneides flavipunctatus</i>	RCT481	CA: Shasta	<i>This study</i>
<i>Aneides flavipunctatus</i>	RLM172	CA: Del Norte	<i>This study</i>
<i>Aneides hardii</i>	MVZ226110	NM: Otero	Vieites et al. 2007
<i>Aneides hardii</i>	RAC020	NM: Lin	<i>This study</i>
<i>Aneides hardii</i>	RAC025	NM: Otero	<i>This study</i>
<i>Aneides hardii</i>	RAC042	NM: Lin	<i>This study</i>
<i>Aneides hardii</i>	RAC054	NM: Lin	<i>This study</i>
<i>Aneides lugubris</i>	MVZ230722	CA: San Diego	<i>This study</i>
<i>Aneides lugubris</i>	MVZ249828	CA: Mariposa	<i>This study</i>
<i>Aneides lugubris</i>	RAC060	CA: Santa Clara	<i>This study</i>
<i>Aneides lugubris</i>	RAC081	CA: Mendocino	<i>This study</i>
<i>Aneides vagrans</i>	HBS26688	CA: Mendocino	<i>This study</i>
<i>Aneides vagrans</i>	MVZ219886	CA: Del Norte	<i>This study</i>
<i>Aneides vagrans</i>	MVZ220991	CA: Humboldt	<i>This study</i>
<i>Aneides vagrans</i>	RAC073	CA: Humboldt	<i>This study</i>
<i>Ensatina escholtzii</i>	MVZ236171	CA: San Luis Obispo	Vieites et al. 2007
<i>Desmognathus fuscus</i>	MVZ224931	MA: Franklin	Vieites et al. 2007
<i>Hydromantes brunus</i>	MVZ238576	CA: Mariposa	Vieites et al. 2007
<i>Plethodon elegans</i>	MVZ220003	CA: Del Norte	Wiens et al. 2005 Vieites et al. 2007

Table 3.2 Individuals used in this study and their voucher numbers, total number of Illumina MiSeq reads, total number of base pairs, total number of contigs used in mitochondrial genome assembly, and sequence length (kb) of mitochondrial genome annotation.

Species	Voucher	Number of reads	Total base pairs	Mitochondrial contigs	Mitochondrial genome (kb)
<i>A. aeneus</i>	DH77584	1,648,165	510,564,223	1	16.8
<i>A. ferreus</i>	RCT545	1,181,785	365,727,056	5	16.4
<i>A. flavipunctatus</i>	MVZ219977	1,165,899	349,989,009	5	17.1
<i>A. flavipunctatus</i>	RLM172	1,044,399	308,615,225	4	17.5
<i>A. hardii</i>	RAC25	931,319	284,267,433	6	18.7
<i>A. hardii</i>	RAC42	1,708,754	507,960,897	5	17.9
<i>A. hardii</i>	RAC54	1,280,531	378,635,736	1	17.0
<i>A. lugubris</i>	MVZ249828	728,185	199,882,489	7	17.0
<i>A. vagrans</i>	MVZ220991	832,332	252,211,876	5	16.5

FIGURES

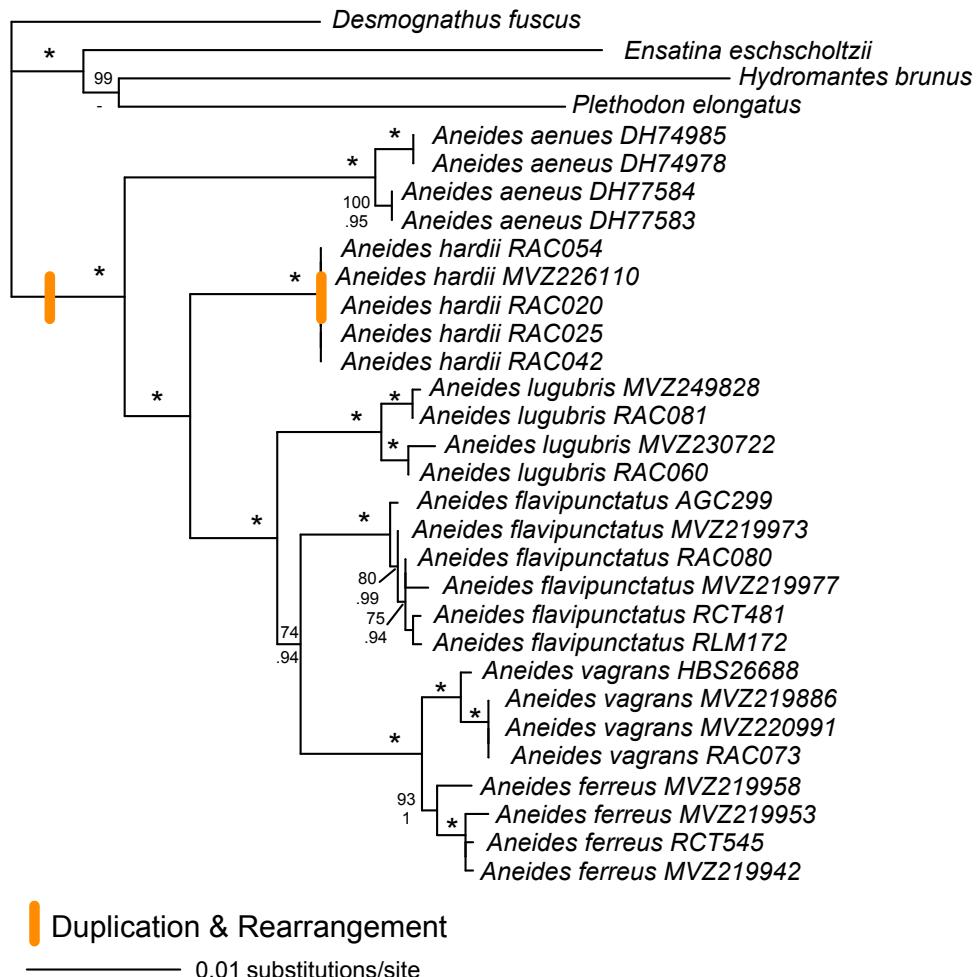


Figure 3.1 Nuclear phylogeny of *Aneides* with nodes labeled with Maximum Likelihood bootstrap support (MLBP) above and Bayesian posterior probabilities (BPP) below. Highly supported internodes (MLBP > 99 and BPP > 0.99) are denoted with an asterisk (*), while weakly supported internodes (MLBP < 70 or BPP < 0.70) are not labeled. *Aneides hardii* branches are all <0.00001. Orange bars indicate inferred mitochondrial duplication and gene rearrangement events.

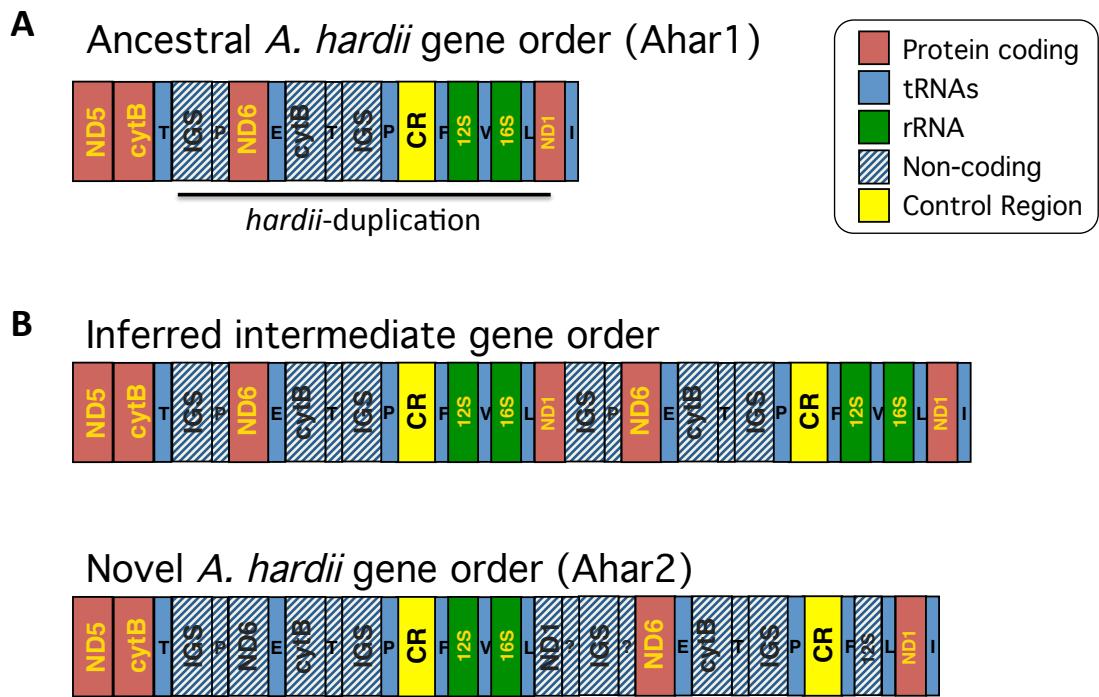


Figure 3.2 Mitochondrial gene rearrangements in *Aneides hardii*. (A) Inferred ancestral *Aneides* mitochondrial gene order (i.e. Ahar1) and hypothesized *hardii*-duplication event. (B) Inferred intermediate gene order after *hardii*-duplication and current novel gene order (i.e. Ahar2).

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CONCLUSION

As the field of evolutionary biology fully enters the genomic era, our ability to collect and analyze genome-scale data and our new opportunities to use these data to create new syntheses across fields have never been greater. Thus, the overall goal of my dissertation research was to study the mechanisms that drive the evolution of mitochondrial genome size, content, and organization in salamanders using a combination of empirical and computational approaches. My dissertation work is an example of the integrative and transformative research that can be done now to gain new insights into long-standing, fundamental questions in biology.

Chapter One analyzes mitochondrial genome sequences of salamanders and frogs, closely related amphibian lineages that differ in metabolic rates, to test for differences in selective constraint on mitochondrial protein-coding genes. With the lowest metabolic requirements among tetrapods, salamanders experience significantly weaker purifying selection on these mitochondrial genes compared to frogs. However, salamanders do not experience weaker selection against genome expansion. These results suggest that different aspects of mitochondrial genome evolution are differently affected by metabolic variation across tetrapod lineages, which adds supports to the complex nature of genomes.

Chapter Two examines the correlation between gene rearrangement/genome expansion and increased rates of substitution in salamander mitochondrial genomes. By comparing rearranged/expanded (i.e. modified) and “normal” mitochondrial genomes, we are able to estimate the mutation gradient in salamander mitochondrial genomes. In modified genomes, we find that genes that move along the gradient are unaffected by their new position because the mutation gradient in salamanders is weak. We also find that levels of selective constraint on

mitochondrial genes do not differ between modified and normal salamander mitochondrial genomes. Together, these results demonstrate that large-scale changes to genome architecture impact rates of mitochondrial gene evolution in predictable ways; however, the interaction between mutation and selection remains complex.

Chapter Three reports the phylogenetic relationships among lineages of *Aneides* based on three nuclear markers and analyzes mitochondrial genome sequences for 11 of the taxa represented in the phylogeny. We describe mitochondrial gene orders for this clade and find support for two independent duplication events within the group, the first rearrangement occurring in the common ancestor of *Aneides* and the second rearrangement existing across different populations of a single species, *A. hardii*. We subsequently estimated levels of mitochondrial sequence divergence and characterized the extent of pseudogene decay for all four *A. hardii* mitochondrial genomes. The results suggest that duplicated protein-coding and rRNA genes are lost more rapidly than other duplicated mitochondrial sequences (i.e. tRNAs, non-coding sequence). Therefore, we conclude that large-scale changes can occur across very shallow levels of genetic divergence, which supports the complex and dynamic nature of genomes.

APPENDIX 1

Table A1.1 Summary of resting metabolic rates from Gatten et al. (Gatten et al. 1992) for frogs and salamander families included in our study.

Order	Family	Species	RMR@15C
Anura	Bombinatoridae	<i>Bufo alvarius</i>	78.7
Anura	Bufonidae	<i>Bufo americanus</i>	110.2
Anura	Bufonidae	<i>Bufo boreas</i>	56.3
Anura	Bufonidae	<i>Bufo bufo</i>	169.3
Anura	Bufonidae	<i>Bufo cognatus</i>	76.2
Anura	Bufonidae	<i>Bufo debilis</i>	128
Anura	Bufonidae	<i>Bufo marinus</i>	37.4
Anura	Bufonidae	<i>Bufo terrestris</i>	80.5
Anura	Bufonidae	<i>Bufo woodhousii</i>	39.5
Anura	Dicroidiidae	<i>Occidozyga martensii</i>	44.8
Anura	Hylidae	<i>Acris crepitans</i>	67
Anura	Hylidae	<i>Cyclorana platycephala</i>	53.7
Anura	Hylidae	<i>Hyla cinerea</i>	126.5
Anura	Hylidae	<i>Hyla gratiosa</i>	89
Anura	Hylidae	<i>Hyla versicolor</i>	104.2
Anura	Hylidae	<i>Phyllomedusa sauvagei</i>	44
Anura	Hylidae	<i>Pseudacris triseriata</i>	225
Anura	Hylidae	<i>Smilisca baudinii</i>	66.8
Anura	Microhylidae	<i>Microhyla carolinensis</i>	66.1
Anura	Pipidae	<i>Xenopus laevis</i>	33.8
Anura	Ranidae	<i>Rana arvalis</i>	96.1
Anura	Ranidae	<i>Rana catesbeiana</i>	57.1
Anura	Ranidae	<i>Rana clamitans</i>	47.3
Anura	Ranidae	<i>Rana erythraea</i>	9.4
Anura	Ranidae	<i>Rana esculenta</i>	88.5
Anura	Ranidae	<i>Rana muscosa</i>	33.7
Anura	Ranidae	<i>Rana palustris</i>	64
Anura	Ranidae	<i>Rana pipiens</i>	51.7
Anura	Ranidae	<i>Rana sylvatica</i>	98.8
Anura	Ranidae	<i>Rana temporaria</i>	67.5
Anura	Ranidae	<i>Rana virgatipes</i>	50
Anura	Scaphiopodidae	<i>Scaphiopus bombifrons</i>	139.2
Anura	Scaphiopodidae	<i>Scaphiopus couchii</i>	84.1
Anura	Scaphiopodidae	<i>Scaphiopus hammondii</i>	93.9
Anura	Scaphiopodidae	<i>Scaphiopus holbrookii</i>	83.7
Caudata	Ambystomatidae	<i>Ambystoma gracile</i>	20.9
Caudata	Ambystomatidae	<i>Ambystoma jeffersonianum</i>	35.4
Caudata	Ambystomatidae	<i>Ambystoma macrodactylum</i>	32.3
Caudata	Ambystomatidae	<i>Ambystoma maculatum</i>	22.9
Caudata	Ambystomatidae	<i>Ambystoma opacum</i>	80
Caudata	Ambystomatidae	<i>Ambystoma tadpoleum</i>	80
Caudata	Ambystomatidae	<i>Ambystoma tigrinum</i>	12.8
Caudata	Amphiumidae	<i>Amphiuma means</i>	6.2

Caudata	Amphiumidae	<i>Amphiuma tridactylum</i>	3.8
Caudata	Cryptobranchidae	<i>Cryptobranchus alleganiensis</i>	20.9
Caudata	Dicamptodontidae	<i>Dicamptodon ensatus</i>	12.9
Caudata	Plethodontidae	<i>Aneides ferreus</i>	27.2
Caudata	Plethodontidae	<i>Aneides flavipunctatus</i>	26.2
Caudata	Plethodontidae	<i>Aneides hardii</i>	50
Caudata	Plethodontidae	<i>Aneides lugubris</i>	26.8
Caudata	Plethodontidae	<i>Batrachoseps attenuatus</i>	41.3
Caudata	Plethodontidae	<i>Bolitoglossa franklini</i>	17.3
Caudata	Plethodontidae	<i>Bolitoglossa morio</i>	21.3
Caudata	Plethodontidae	<i>Bolitoglossa subpalmata</i>	31.5
Caudata	Plethodontidae	<i>Chiropterotriton bromeliacia</i>	24.4
Caudata	Plethodontidae	<i>Desmognathus fuscus</i>	34.4
Caudata	Plethodontidae	<i>Desmognathus monticola</i>	57
Caudata	Plethodontidae	<i>Desmognathus ochrophaeus</i>	36
Caudata	Plethodontidae	<i>Desmognathus quadromaculatus</i>	19.3
Caudata	Plethodontidae	<i>Ensatina eschscholtzii</i>	38.1
Caudata	Plethodontidae	<i>Eurycea bislineata</i>	45.9
Caudata	Plethodontidae	<i>Eurycea longicauda</i>	32.1
Caudata	Plethodontidae	<i>Eurycea multiplicata</i>	21.8
Caudata	Plethodontidae	<i>Gyrinophilus danieli</i>	22.3
Caudata	Plethodontidae	<i>Gyrinophilus porphyriticus</i>	58
Caudata	Plethodontidae	<i>Hydromantes sp</i>	38.4
Caudata	Plethodontidae	<i>Plethodon cinereus</i>	40
Caudata	Plethodontidae	<i>Plethodon glutinosus</i>	33.9
Caudata	Plethodontidae	<i>Plethodon jordani</i>	27.9
Caudata	Plethodontidae	<i>Plethodon neomexicanus</i>	55
Caudata	Plethodontidae	<i>Pseudoeurycea belli</i>	17.2
Caudata	Plethodontidae	<i>Pseudoeurycea brunnata</i>	19.7
Caudata	Plethodontidae	<i>Pseudoeurycea cephalica</i>	24.4
Caudata	Plethodontidae	<i>Pseudoeurycea cochranae</i>	20.5
Caudata	Plethodontidae	<i>Pseudoeurycea gadovii</i>	29.5
Caudata	Plethodontidae	<i>Pseudoeurycea goebeli</i>	22.7
Caudata	Plethodontidae	<i>Pseudoeurycea leprosa</i>	22.6
Caudata	Plethodontidae	<i>Pseudoeurycea rex</i>	21.1
Caudata	Plethodontidae	<i>Pseudoeurycea smithii</i>	17.8
Caudata	Plethodontidae	<i>Pseudotriton ruber</i>	22.5
Caudata	Plethodontidae	<i>Thorius sp</i>	25.8
Caudata	Proteidae	<i>Neturus maculosus</i>	11.2
Caudata	Rhyacotritonidae	<i>Rhyacotriton olympicus</i>	96
Caudata	Salamandridae	<i>Notophthalmus viridescens</i>	34.4
Caudata	Salamandridae	<i>Salamandra salamandra</i>	81
Caudata	Salamandridae	<i>Taricha granulosa</i>	24.4
Caudata	Salamandridae	<i>Taricha rivularis</i>	27.8
Caudata	Salamandridae	<i>Taricha torosa</i>	30.1
Caudata	Sirenidae	<i>Siren lacertina</i>	6.6

Table A1.2 Summary of NCBI Genbank accession numbers and ω estimates for mitochondrial and nuclear genes for all taxa included in this study. Tip lineage ω estimates are listed by taxon. Internal branch ω estimates are listed by nodes, which are indicated as numbers.

Order	Family	Species	Genbank	Omega
Anura	Alytidae	<i>Alytes obstetricans</i>	AY583334	0.0488
Anura	Alytidae	<i>Discoglossus galganoi</i>	AY583338	0.0705
Anura	Bombinatoridae	<i>Bombina orientalis</i>	AY323756	0.1383
Anura	Bombinatoridae	<i>Bombina variegata</i>	AY523750	0.0001
Anura	Bufonidae	<i>Bufo americanus</i>	DQ158352	0.0001
Anura	Bufonidae	<i>Bufo boreas</i>	DQ158360	0.6656
Anura	Bufonidae	<i>Bufo bufo</i>	EU497611	0.1096
Anura	Bufonidae	<i>Bufo cognatus</i>	DQ158367	0.0001
Anura	Bufonidae	<i>Bufo debilis</i>	DQ158371	0.0001
Anura	Bufonidae	<i>Bufo marinus</i>	DQ158393	0.0001
Anura	Bufonidae	<i>Bufo melanostictus</i>	EU712821	0.0001
Anura	Bufonidae	<i>Bufo woodhousii</i>	DQ158413	0.0001
Anura	Dic平glossidae	<i>Euphlyctis cyanophlyctis</i>	DQ347205	0.0791
Anura	Dic平glossidae	<i>Fejervarya syhadrensis</i>	DQ347203	0.0476
Anura	Dic平glossidae	<i>Hoplobatrachus occipitalis</i>	DQ347217	0.0001
Anura	Dic平glossidae	<i>Limnonectes kuhlii</i>	DQ347232	0.0397
Anura	Dic平glossidae	<i>Limnonectes limborgi</i>	DQ347286	0.1258
Anura	Hylidae	<i>Acris crepitans</i>	EF107304	0.0742
Anura	Hylidae	<i>Hyla arborea</i>	FJ227083	0.0001
Anura	Hylidae	<i>Hyla cinerea</i>	FJ227076	0.0662
Anura	Hylidae	<i>Hyla japonica</i>	FJ227068	0.0001
Anura	Hylidae	<i>Smilisca baudinii</i>	DQ830932	0.0001
Anura	Leiopelmatidae	<i>Leiopelma archeyi</i>	AY523748	0.0874
Anura	Mantellidae	<i>Mantella madagascariensis</i>	DQ019500	0.0390
Anura	Microhylidae	<i>Kaloula pulchra</i>	EF396091	0.0001
Anura	Microhylidae	<i>Microhyla heymonsi</i>	EF396095	0.0193
Anura	Microhylidae	<i>Microhyla ornata</i>	AY364198	0.1852
Anura	Microhylidae	<i>Microhyla pulchra</i>	EF396093	0.0371
Anura	Dic平glossidae	<i>Occidozyga lima</i>	DQ019503	0.1045
Anura	Pelobatidae	<i>Pelobates cultripes</i>	AY323758	0.0533
Anura	Pipidae	<i>Xenopus laevis</i>	EF535914	0.1102
Anura	Pipidae	<i>Xenopus tropicalis</i>	AY874307	0.0612
Anura	Ranidae	<i>Amolops marmoratus</i>	EF088241	0.2163
Anura	Ranidae	<i>Rana boylei</i>	DQ347277	0.0001
Anura	Ranidae	<i>Rana clamitans</i>	DQ347262	0.6611
Anura	Ranidae	<i>Rana pipiens</i>	DQ347265	0.1630
Anura	Ranidae	<i>Rana sylvatica</i>	DQ019511	0.1627
Anura	Rhacophoridae	<i>Buergeria buergeri</i>	AY948921	0.0734
Anura	Scaphiopodidae	<i>Scaphiopus couchii</i>	AY323759	0.0288
Anura	Scaphiopodidae	<i>Spea multiplicata</i>	AY523749	0.0514
Caudata	Ambystomatidae	<i>Ambystoma gracile</i>	AY650131	0.0001
Caudata	Ambystomatidae	<i>Ambystoma mexicanum</i>	AY323752	0.0001
Caudata	Ambystomatidae	<i>Ambystoma opacum</i>	AY650130	0.1262
Caudata	Ambystomatidae	<i>Ambystoma ordinarium</i>	AY583345	0.6503
Caudata	Amphiumidae	<i>Amphiuma means</i>	AY650127	0.0618

Caudata	Cryptobranchidae	<i>Andrias davidianus</i>	AY650142	-
Caudata	Cryptobranchidae	<i>Andrias japonicus</i>	AY583346	0.0001
Caudata	Cryptobranchidae	<i>Cryptobranchus alleganiensis</i>	AY650141	-
Caudata	Dicamptodontidae	<i>Dicamptodon ensatus</i>	EF107335	0.0001
Caudata	Dicamptodontidae	<i>Dicamptodon tenebrosus</i>	EU275789	0.0001
Caudata	Hynobiidae	<i>Batrachuperus pinchonii</i>	EF018054	0.3444
Caudata	Hynobiidae	<i>Hynobius formosanus</i>	DQ347285	0.0001
Caudata	Hynobiidae	<i>Hynobius nebulosus</i>	AY650144	0.0804
Caudata	Hynobiidae	<i>Onychodactylus japonicus</i>	AY583350	0.1138
Caudata	Hynobiidae	<i>Salamandrella keyserlingii</i>	AY650145	0.3249
Caudata	Plethodontidae	<i>Aneides aeneus</i>	AY691701	0.0448
Caudata	Plethodontidae	<i>Aneides hardii</i>	EU275780	0.0001
Caudata	Plethodontidae	<i>Aneides lugubris</i>	EU275807	0.0433
Caudata	Plethodontidae	<i>Batrachoseps attenuatus</i>	EU020160	0.0001
Caudata	Plethodontidae	<i>Batrachoseps wrighti</i>	EU020165	0.0001
Caudata	Plethodontidae	<i>Bolitoglossa helmrichi</i>	AY650124	1.0298
Caudata	Plethodontidae	<i>Bolitoglossa mexicana</i>	EF018055	0.1634
Caudata	Plethodontidae	<i>Desmognathus fuscus</i>	EU275781	0.1053
Caudata	Plethodontidae	<i>Desmognathus wrighti</i>	AY691699	0.0001
Caudata	Plethodontidae	<i>Ensatina eschscholtzii</i>	EU275785	0.0001
Caudata	Plethodontidae	<i>Eurycea bislineata</i>	EU275784	0.0417
Caudata	Plethodontidae	<i>Eurycea longicauda</i>	AY650121	0.0028
Caudata	Plethodontidae	<i>Gyrinophilus porphyriticus</i>	AY583349	0.3186
Caudata	Plethodontidae	<i>Hemidactylum scutatum</i>	AY691712	0.1423
Caudata	Plethodontidae	<i>Hydromantes brunus</i>	EU275790	-
Caudata	Plethodontidae	<i>Hydromantes platycephalus</i>	EU275793	0.0001
Caudata	Plethodontidae	<i>Karsenia koreana</i>	AY887135	0.1125
Caudata	Plethodontidae	<i>Phaeognathus hubrichti</i>	EU275783	0.0756
Caudata	Plethodontidae	<i>Plethodon cinereus</i>	DQ995021	0.0612
Caudata	Plethodontidae	<i>Plethodon elongatus</i>	AY650120	0.4384
Caudata	Plethodontidae	<i>Plethodon neomexicanus</i>	DQ995044	0.0001
Caudata	Plethodontidae	<i>Pseudoeurycea rex</i>	AY650125	0.1958
Caudata	Plethodontidae	<i>Pseudotriton ruber</i>	AY650123	0.1909
Caudata	Plethodontidae	<i>Stereochilus marginatus</i>	AY691713	0.1052
Caudata	Proteidae	<i>Necturus maculosus</i>	AY650137	0.0661
Caudata	Proteidae	<i>Proteus anguinus</i>	AY650138	0.0592
Caudata	Rhyacotritonidae	<i>Rhyacotriton variegatus</i>	AY691693	0.1290
Caudata	Salamandridae	<i>Lyciasalamandra luschani</i>	AY323753	0.0001
Caudata	Salamandridae	<i>Notophthalmus viridescens</i>	AY650134	0.0001
Caudata	Salamandridae	<i>Pachytriton labiatus</i>	AY583351	0.1861
Caudata	Salamandridae	<i>Pleurodeles poireti</i>	EU275787	0.0001
Caudata	Salamandridae	<i>Pleurodeles waltl</i>	AY523736	0.0001
Caudata	Salamandridae	<i>Salamandra salamandra</i>	AY583352	-
Caudata	Salamandridae	<i>Taricha rivularis</i>	AY650133	-
Caudata	Salamandridae	<i>Taricha torosa</i>	EF107340	0.0001
Caudata	Salamandridae	<i>Triturus marmoratus</i>	AY583354	0.0886
Caudata	Salamandridae	<i>Tylototriton wenxianensis</i>	EU275788	0.0001
Caudata	Sirenidae	<i>Siren intermedia</i>	AY583353	0.0001
Caudata	Sirenidae	<i>Siren lacertian</i>	EF107307	0.0001

APPENDIX 2

Table A2.1 Summary of NCBI Genbank numbers and genome characteristics for 62 salamander mitochondrial genomes analyzed in our study.

Genbank	Species	mtG Size	A%	T%	G%	C%	General ATSkew	General GCSkew	AT Skew	GC Skew	p4_AT Skew	p4_GC Skew
NC006888	<i>Ambystoma andersoni</i>	16370	0.35	0.32	0.13	0.20	0.04	-0.21	-0.01	-0.25	0.30	-0.41
NC014568	<i>Ambystoma barbouri</i>	16365	0.34	0.31	0.14	0.21	0.04	-0.20	-0.01	-0.25	0.34	-0.42
NC006890	<i>Ambystoma californiense</i>	16374	0.34	0.32	0.14	0.20	0.04	-0.19	-0.02	-0.24	0.28	-0.31
NC006889	<i>Ambystoma dumerilii</i>	16370	0.35	0.32	0.13	0.20	0.04	-0.20	-0.02	-0.24	0.30	-0.46
NC006330	<i>Ambystoma laterale</i>	16367	0.34	0.32	0.14	0.21	0.04	-0.20	-0.02	-0.25	0.31	-0.41
NC005797	<i>Ambystoma mexicanum</i>	16369	0.35	0.32	0.13	0.20	0.04	-0.21	-0.01	-0.25	0.30	-0.42
NC014571	<i>Ambystoma texanum</i>	16367	0.35	0.32	0.13	0.20	0.04	-0.21	-0.01	-0.25	0.32	-0.41
NC006887	<i>Ambystoma tigrinum</i>	16375	0.35	0.32	0.13	0.20	0.04	-0.19	-0.02	-0.24	0.30	-0.40
NC004926	<i>Andrias davidianus</i>	16503	0.32	0.33	0.14	0.21	-0.01	-0.19	-0.08	-0.23	0.16	-0.54
NC007446	<i>Andrias japonicus</i>	16298	0.32	0.33	0.14	0.21	-0.01	-0.18	-0.07	-0.22	0.17	-0.48
NC006327	<i>Aneides flavipunctatus</i>	20197	0.34	0.33	0.13	0.21	0.02	-0.24	-0.05	-0.25	0.24	-0.62
NC006338	<i>Aneides hardii</i>	22184	0.34	0.31	0.13	0.23	0.06	-0.28	-0.03	-0.28	0.32	-0.66
NC006340	<i>Batrachoseps attenuatus</i>	17559	0.34	0.30	0.14	0.21	0.06	-0.20	-0.01	-0.26	0.37	-0.57
NC006333	<i>Batrachoseps wrighti</i>	19789	0.34	0.30	0.13	0.22	0.07	-0.25	0.03	-0.28	0.47	-0.54
NC008077	<i>Batrachuperus londongensis</i>	16379	0.34	0.33	0.13	0.19	0.01	-0.18	-0.05	-0.22	0.30	-0.44

NC008083	Batrachuperus pinchonii	16390	0.34	0.33	0.13	0.20	0.02	-0.19	-0.04	-0.22	0.32	-0.52
NC008085	Batrachuperus tibetanus	16379	0.34	0.33	0.14	0.20	0.02	-0.18	-0.04	-0.22	0.33	-0.51
NC012430	Batrachuperus yenyuanensis	16394	0.34	0.33	0.14	0.19	0.01	-0.16	-0.05	-0.19	0.31	-0.43
NC006346	Bolitoglossa n. sp.	21657	0.33	0.31	0.12	0.24	0.02	-0.32	0.00	-0.30	0.37	-0.60
NC006339	Desmognathus fuscus	16628	0.36	0.33	0.12	0.19	0.03	-0.21	-0.02	-0.24	0.34	-0.62
NC006337	Desmognathus wrighti	16578	0.35	0.31	0.13	0.20	0.05	-0.21	-0.01	-0.24	0.44	-0.57
NC017870	Echinotriton andersoni	16268	0.34	0.27	0.14	0.25	0.13	-0.29	0.11	-0.33	0.52	-0.53
NC006328	Ensatina eschscholtzii	22816	0.35	0.32	0.12	0.21	0.04	-0.29	-0.01	-0.33	0.31	-0.80
NC006329	Eurycea bislineata	17184	0.33	0.31	0.13	0.23	0.03	-0.26	-0.02	-0.30	0.31	-0.69
NC006341	Gyrinophilus porphyriticus	16778	0.34	0.28	0.14	0.24	0.10	-0.28	0.04	-0.34	0.47	-0.73
NC006342	Hemidactylum scutatum	17421	0.34	0.30	0.15	0.22	0.07	-0.19	0.01	-0.26	0.39	-0.45
NC006345	Hydromantes brunus	17220	0.35	0.35	0.12	0.19	0.00	-0.21	-0.05	-0.26	0.18	-0.79
NC008076	Hynobius amjiensis	16401	0.33	0.32	0.14	0.21	0.02	-0.20	-0.03	-0.24	0.34	-0.46
NC009335	Hynobius arisanensis	16401	0.34	0.33	0.13	0.20	0.02	-0.21	-0.03	-0.25	0.33	-0.57
NC008088	Hynobius chinensis	16408	0.33	0.32	0.14	0.21	0.03	-0.21	-0.02	-0.25	0.36	-0.45
NC008084	Hynobius formosanus	16394	0.34	0.33	0.13	0.20	0.02	-0.21	-0.03	-0.25	0.33	-0.62
NC013762	Hynobius guabangshanensis	16408	0.33	0.32	0.14	0.21	0.03	-0.21	-0.02	-0.25	0.36	-0.45
NC008079	Hynobius leechii	16428	0.34	0.33	0.14	0.20	0.02	-0.19	-0.04	-0.23	0.32	-0.51
NC010224	Hynobius quelpaertensis	16407	0.33	0.32	0.14	0.21	0.02	-0.20	-0.04	-0.24	0.34	-0.53
NC013825	Hynobius yangi	16424	0.33	0.32	0.14	0.20	0.02	-0.19	-0.04	-0.22	0.30	-0.47
NC008078	Liua shihi	16376	0.34	0.32	0.13	0.20	0.02	-0.20	-0.03	-0.23	0.30	-0.50

NC008081	<i>Liua tsinpaensis</i>	16380	0.33	0.33	0.14	0.20	0.01	-0.19	-0.05	-0.22	0.25	-0.46
NC002756	<i>Lyciasalamandra atifi</i>	16650	0.32	0.29	0.14	0.24	0.05	-0.25	0.00	-0.28	0.37	-0.43
NC006326	<i>Oedipina poelzi</i>	16731	0.36	0.28	0.14	0.23	0.12	-0.24	0.08	-0.28	0.56	-0.43
NC008089	<i>Onychodactylus fischeri</i>	16456	0.35	0.34	0.13	0.19	0.02	-0.19	-0.04	-0.21	0.26	-0.57
NC008080	<i>Pachyhynobius shangchengensis</i>	16394	0.34	0.33	0.13	0.20	0.02	-0.20	-0.04	-0.24	0.30	-0.61
NC008091	<i>Paradactyodon gorganensis</i>	16374	0.34	0.32	0.14	0.20	0.02	-0.20	-0.04	-0.24	0.30	-0.52
NC008090	<i>Paradactyodon mustersi</i>	16383	0.33	0.32	0.14	0.20	0.02	-0.20	-0.04	-0.24	0.30	-0.50
NC006407	<i>Paramesotriton hongkongensis</i>	16285	0.33	0.28	0.15	0.24	0.08	-0.24	0.02	-0.27	0.43	-0.30
NC006344	<i>Phaeognathus hubrichti</i>	16294	0.34	0.32	0.13	0.21	0.04	-0.25	-0.02	-0.29	0.32	-0.78
NC006343	<i>Plethodon cinereus</i>	20001	0.34	0.30	0.13	0.24	0.06	-0.30	-0.01	-0.33	0.33	-0.66
NC006335	<i>Plethodon elongatus</i>	18767	0.34	0.30	0.13	0.23	0.06	-0.27	0.00	-0.33	0.34	-0.78
NC006334	<i>Plethodon petraeus</i>	19235	0.34	0.30	0.13	0.23	0.06	-0.30	0.01	-0.35	0.40	-0.68
NC006332	<i>Pseudotriton ruber</i>	16661	0.34	0.29	0.13	0.23	0.08	-0.26	0.02	-0.31	0.39	-0.69
NC004021	<i>Ranodon sibiricus</i>	16418	0.35	0.33	0.13	0.19	0.03	-0.19	-0.02	-0.23	0.37	-0.55
NC006331	<i>Rhyacotriton variegatus</i>	21606	0.35	0.33	0.12	0.20	0.04	-0.23	-0.02	-0.22	0.31	-0.51
NC008082	<i>Salamandrella keyserlingii</i>	16338	0.33	0.32	0.14	0.21	0.02	-0.19	-0.04	-0.22	0.31	-0.47
NC006325	<i>Stereochilus marginatus</i>	19631	0.35	0.26	0.14	0.25	0.15	-0.28	0.03	-0.33	0.43	-0.60
NC006336	<i>Thorius n. sp.</i>	19097	0.33	0.28	0.15	0.23	0.07	-0.21	0.01	-0.28	0.45	-0.46
NC015788	<i>Triturus carnifex</i>	16560	0.31	0.27	0.16	0.26	0.07	-0.24	0.01	-0.28	0.41	-0.38
NC015790	<i>Triturus cristatus</i>	16564	0.31	0.27	0.16	0.26	0.07	-0.23	0.01	-0.27	0.42	-0.39
NC015791	<i>Triturus dobrogicus</i>	16425	0.31	0.28	0.16	0.25	0.06	-0.23	0.00	-0.27	0.40	-0.38
NC015792	<i>Triturus karelinii</i>	16541	0.31	0.28	0.16	0.25	0.06	-0.23	0.00	-0.26	0.39	-0.39

NC015794	Triturus macedonicus	16569	0.31	0.27	0.16	0.26	0.07	-0.24	0.00	-0.27	0.40	-0.40
NC015795	Triturus marmoratus	16546	0.32	0.27	0.15	0.26	0.08	-0.26	0.02	-0.30	0.43	-0.49
NC015796	Triturus pygmaeus	16442	0.32	0.27	0.15	0.26	0.08	-0.26	0.02	-0.30	0.44	-0.49
NC017871	Tylototriton verrucosus	17100	0.34	0.25	0.14	0.26	0.15	-0.30	0.13	-0.33	0.53	-0.49

Table A2.2 Summary of mitochondrial genome modification (0 = normal, 1 = modified), genome characteristics, substitution rates, and ω estimates for each gene for 62 salamander mitochondrial genomes analyzed in our study.

Species	mtG	Genome Size	Gene	Start (bp)	Stop (bp)	DssH start	DssH stop	DssH average	mutPos bp	dS	dN	Omega a
Ambystoma andersoni	0	16370	ATP6	7918	8601	0.340	0.423	0.382	6245	0.009932	0.000001	--
Ambystoma andersoni	0	16370	ATP8	7760	7927	0.321	0.341	0.331	5414	0.000005	0	--
Ambystoma andersoni	0	16370	COX1	5311	6858	0.021	0.210	0.116	1896	0.018831	0.003769	0.2001
Ambystoma andersoni	0	16370	COX2	6999	7683	0.228	0.311	0.269	4408	0.015591	0.008403	0.5389
Ambystoma andersoni	0	16370	COX3	8601	9384	0.423	0.519	0.471	7711	0.015822	0.00175	0.1106
Ambystoma andersoni	0	16370	CYTB	14109	15249	1.096	1.235	1.166	19084	0.006562	0.00131	0.1997
Ambystoma andersoni	0	16370	ND1	2699	3670	0.702	0.821	0.761	12465	0.007018	0.003176	0.4525
Ambystoma andersoni	0	16370	ND2	3881	4924	0.847	0.974	0.910	14901	0.030698	0.00542	0.1766
Ambystoma andersoni	0	16370	ND3	9454	9801	0.527	0.570	0.549	8981	0.037491	0.018239	0.4865
Ambystoma andersoni	0	16370	ND4	10159	11533	0.614	0.781	0.698	11418	0.033415	0.001126	0.0337
Ambystoma andersoni	0	16370	ND4L	9869	10165	0.578	0.614	0.596	9760	0.000005	0	--
Ambystoma andersoni	0	16370	ND5	11744	13543	0.807	1.027	0.917	15013	0.009536	0.001616	0.1694
Ambystoma andersoni	0	16370	ND6	13526	14038	1.025	1.088	1.056	17290	0.007839	0.000001	0.0001
Ambystoma barbouri	0	16365	ATP6	7910	8593	0.339	0.423	0.381	6231	0.223672	0.00201	0.0001
Ambystoma barbouri	0	16365	ATP8	7752	7919	0.320	0.340	0.330	5399	0.01852	0.000002	0.0001
Ambystoma barbouri	0	16365	COX1	5302	6849	0.020	0.209	0.115	1879	0.166124	0.000017	0.0001
Ambystoma barbouri	0	16365	COX2	6991	7676	0.227	0.310	0.269	4395	0.182152	0.004072	0.0224

<i>Ambystoma</i> <i>barbouri</i>	0	16365	COX3	8593	9377	0.423	0.518	0.470	7698	0.111579	0.001623	0.0145
<i>Ambystoma</i> <i>barbouri</i>	0	16365	CYTB	14100	15240	1.096	1.235	1.165	19068	0.267031	0.002205	0.0083
<i>Ambystoma</i> <i>barbouri</i>	0	16365	ND1	2693	3661	0.701	0.820	0.761	12446	0.24437	0.005753	0.0235
<i>Ambystoma</i> <i>barbouri</i>	0	16365	ND2	3870	4913	0.845	0.973	0.909	14876	0.217157	0.00347	0.016
<i>Ambystoma</i> <i>barbouri</i>	0	16365	ND3	9443	9793	0.526	0.569	0.548	8964	0.235931	0.004873	0.0207
<i>Ambystoma</i> <i>barbouri</i>	0	16365	ND4	10151	11525	0.613	0.781	0.697	11404	0.174574	0.005882	0.0337
<i>Ambystoma</i> <i>barbouri</i>	0	16365	ND4L	9861	10157	0.578	0.614	0.596	9746	0.207964	0.008682	0.0417
<i>Ambystoma</i> <i>barbouri</i>	0	16365	ND5	11735	13534	0.807	1.026	0.916	14997	0.204058	0.004612	0.0226
<i>Ambystoma</i> <i>barbouri</i>	0	16365	ND6	13517	14032	1.024	1.087	1.056	17277	0.172561	0.000017	0.0001
<i>Ambystoma</i> <i>californiense</i>	0	16374	ATP6	7921	8604	0.340	0.424	0.382	6252	0.093099	0.006419	--
<i>Ambystoma</i> <i>californiense</i>	0	16374	ATP8	7763	7930	0.321	0.341	0.331	5420	0.000017	0.016826	--
<i>Ambystoma</i> <i>californiense</i>	0	16374	COX1	5314	6861	0.022	0.211	0.116	1901	0.196244	0.006539	0.0333
<i>Ambystoma</i> <i>californiense</i>	0	16374	COX2	7002	7686	0.228	0.311	0.270	4414	0.153043	0.000015	0.0001
<i>Ambystoma</i> <i>californiense</i>	0	16374	COX3	8604	9387	0.424	0.519	0.471	7717	0.095444	0.001763	0.0185
<i>Ambystoma</i> <i>californiense</i>	0	16374	CYTB	14112	15252	1.096	1.236	1.166	19090	0.258746	0.004548	0.0176
<i>Ambystoma</i> <i>californiense</i>	0	16374	ND1	2698	3669	0.702	0.821	0.761	12467	0.239416	0.005706	0.0238
<i>Ambystoma</i> <i>californiense</i>	0	16374	ND2	3881	4924	0.847	0.974	0.910	14905	0.202118	0.006684	0.0331
<i>Ambystoma</i> <i>californiense</i>	0	16374	ND3	9457	9804	0.528	0.570	0.549	8988	0.011156	0.004039	0.362
<i>Ambystoma</i> <i>californiense</i>	0	16374	ND4	10162	11536	0.614	0.782	0.698	11424	0.174686	0.011606	0.0664

<i>Ambystoma californiense</i>	0	16374	ND4L	9872	10168	0.578	0.615	0.596	9766	0.053525	0.000005	0.0001
<i>Ambystoma californiense</i>	0	16374	ND5	11747	13546	0.807	1.027	0.917	15019	0.274036	0.007131	0.026
<i>Ambystoma californiense</i>	0	16374	ND6	13529	14041	1.025	1.088	1.056	17296	0.136929	0.008732	0.0638
<i>Ambystoma dumerilii</i>	0	16370	ATP6	7918	8601	0.340	0.423	0.382	6247	0.079566	0.004222	0.5624
<i>Ambystoma dumerilii</i>	0	16370	ATP8	7760	7927	0.321	0.341	0.331	5415	0.05927	0.033332	0.5624
<i>Ambystoma dumerilii</i>	0	16370	COX1	5311	6858	0.021	0.210	0.116	1897	0.088769	0.00084	0.0095
<i>Ambystoma dumerilii</i>	0	16370	COX2	6999	7683	0.228	0.311	0.269	4410	0.13221	0.000013	0.0001
<i>Ambystoma dumerilii</i>	0	16370	COX3	8601	9384	0.423	0.519	0.471	7713	0.075798	0.000008	0.0001
<i>Ambystoma dumerilii</i>	0	16370	CYTB	14109	15249	1.096	1.236	1.166	19087	0.117531	0.001106	0.0094
<i>Ambystoma dumerilii</i>	0	16370	ND1	2698	3669	0.702	0.821	0.761	12465	0.140331	0.011733	0.0836
<i>Ambystoma dumerilii</i>	0	16370	ND2	3880	4923	0.847	0.974	0.910	14901	0.122272	0.005292	0.0433
<i>Ambystoma dumerilii</i>	0	16370	ND3	9454	9801	0.528	0.570	0.549	8983	0.000016	0.016249	--
<i>Ambystoma dumerilii</i>	0	16370	ND4	10159	11533	0.614	0.782	0.698	11421	0.110523	0.001086	0.0098
<i>Ambystoma dumerilii</i>	0	16370	ND4L	9869	10165	0.578	0.614	0.596	9762	0.000005	0	--
<i>Ambystoma dumerilii</i>	0	16370	ND5	11744	13543	0.807	1.027	0.917	15015	0.075694	0.011492	0.1518
<i>Ambystoma dumerilii</i>	0	16370	ND6	13526	14038	1.025	1.088	1.056	17292	0.051611	0.000005	0.0001
<i>Ambystoma laterale</i>	0	16367	ATP6	7916	8599	0.339	0.422	0.380	6227	0.443176	0.010115	--
<i>Ambystoma laterale</i>	0	16367	ATP8	7758	7925	0.319	0.340	0.330	5395	0.000005	0	--
<i>Ambystoma laterale</i>	0	16367	COX1	5309	6856	0.020	0.209	0.115	1877	0.506299	0.000851	0.0017
<i>Ambystoma laterale</i>	0	16367	COX2	6997	7682	0.226	0.310	0.268	4390	0.192355	0.000019	0.0001
<i>Ambystoma laterale</i>	0	16367	COX3	8599	9382	0.422	0.518	0.470	7693	0.321671	0.004388	0.0136

<i>Ambystoma laterale</i>	0	16367	CYTB	14107	15247	1.095	1.235	1.165	19067	0.353623	0.00736	0.0208
<i>Ambystoma laterale</i>	0	16367	ND1	2692	3660	0.700	0.819	0.760	12432	0.394727	0.007332	0.0186
<i>Ambystoma laterale</i>	0	16367	ND2	3878	4921	0.845	0.973	0.909	14878	0.306331	0.011671	0.0381
<i>Ambystoma laterale</i>	0	16367	ND3	9449	9799	0.526	0.569	0.547	8960	0.000005	0	--
<i>Ambystoma laterale</i>	0	16367	ND4	10157	11525	0.613	0.780	0.696	11394	0.198263	0.007382	0.0372
<i>Ambystoma laterale</i>	0	16367	ND4L	9867	10163	0.577	0.613	0.595	9742	0.044376	0.000004	0.0001
<i>Ambystoma laterale</i>	0	16367	ND5	11742	13538	0.806	1.026	0.916	14992	0.271178	0.011339	0.0418
<i>Ambystoma laterale</i>	0	16367	ND6	13541	14039	1.026	1.087	1.057	17292	0.000005	0	--
<i>Ambystoma mexicanum</i>	0	16369	ATP6	7917	8600	0.339	0.422	0.380	6227	0.006767	0.000001	0.0001
<i>Ambystoma mexicanum</i>	0	16369	ATP8	7759	7926	0.319	0.340	0.330	5395	0.050142	0.000005	0.0001
<i>Ambystoma mexicanum</i>	0	16369	COX1	5310	6857	0.020	0.209	0.115	1878	0.020416	0.000002	0.0001
<i>Ambystoma mexicanum</i>	0	16369	COX2	6998	7683	0.226	0.310	0.268	4391	0.008724	0.007404	0.8487
<i>Ambystoma mexicanum</i>	0	16369	COX3	8600	9383	0.422	0.518	0.470	7693	0.018037	0.000002	0.0001
<i>Ambystoma mexicanum</i>	0	16369	CYTB	14109	15249	1.095	1.235	1.165	19067	0.008248	0.000001	0.0001
<i>Ambystoma mexicanum</i>	0	16369	ND1	2698	3666	0.701	0.819	0.760	12443	0.007139	0.000001	0.0001
<i>Ambystoma mexicanum</i>	0	16369	ND2	3880	4923	0.845	0.973	0.909	14882	0.002358	0.003387	1.4363
<i>Ambystoma mexicanum</i>	0	16369	ND3	9453	9800	0.526	0.569	0.548	8964	0.068881	0.004743	0.0689
<i>Ambystoma mexicanum</i>	0	16369	ND4	10158	11532	0.613	0.780	0.696	11400	0.000001	0.001149	--
<i>Ambystoma mexicanum</i>	0	16369	ND4L	9868	10164	0.577	0.613	0.595	9742	0.029768	0.000003	0.0001
<i>Ambystoma mexicanum</i>	0	16369	ND5	11743	13539	0.806	1.026	0.916	14992	0.010574	0.000001	0.0001
<i>Ambystoma mexicanum</i>	0	16369	ND6	13525	14040	1.024	1.087	1.055	17275	0.05082	0.004178	0.0822
<i>Ambystoma texanum</i>	0	16367	ATP6	7911	8594	0.339	0.422	0.381	6231	0.105586	0.002031	0.0941

Ambystoma texanum	0	16367	ATP8	7753	7920	0.320	0.340	0.330	5399	0.153964	0.01449	0.0941
Ambystoma texanum	0	16367	COX1	5303	6850	0.020	0.209	0.115	1879	0.261263	0.000847	0.0032
Ambystoma texanum	0	16367	COX2	6992	7677	0.227	0.310	0.269	4395	0.133518	0.001973	0.0148
Ambystoma texanum	0	16367	COX3	8594	9378	0.422	0.518	0.470	7697	0.245898	0.003337	0.0136
Ambystoma texanum	0	16367	CYTB	14102	15242	1.096	1.235	1.165	19070	0.212609	0.001099	0.0052
Ambystoma texanum	0	16367	ND1	2693	3661	0.701	0.820	0.761	12447	0.205821	0.00286	0.0139
Ambystoma texanum	0	16367	ND2	3872	4913	0.845	0.973	0.909	14878	0.252632	0.003113	0.0123
Ambystoma texanum	0	16367	ND3	9445	9795	0.526	0.569	0.548	8966	0.227102	0.010168	0.0448
Ambystoma texanum	0	16367	ND4	10153	11527	0.613	0.781	0.697	11405	0.211521	0.007321	0.0346
Ambystoma texanum	0	16367	ND4L	9863	10159	0.578	0.614	0.596	9748	0.016848	0.000002	0.0001
Ambystoma texanum	0	16367	ND5	11737	13536	0.807	1.026	0.916	14999	0.228572	0.004046	0.0177
Ambystoma texanum	0	16367	ND6	13519	14034	1.024	1.087	1.056	17279	0.154031	0.000015	0.0001
Ambystoma tigrinum	0	16375	ATP6	7922	8605	0.340	0.423	0.382	6247	0.179085	0.006179	--
Ambystoma tigrinum	0	16375	ATP8	7764	7931	0.321	0.341	0.331	5415	0.000005	0	--
Ambystoma tigrinum	0	16375	COX1	5315	6862	0.021	0.210	0.116	1897	0.106601	0.000011	0.0001
Ambystoma tigrinum	0	16375	COX2	7003	7688	0.228	0.311	0.269	4411	0.067361	0.000007	0.0001
Ambystoma tigrinum	0	16375	COX3	8605	9389	0.423	0.519	0.471	7714	0.087717	0.001541	0.0176
Ambystoma tigrinum	0	16375	CYTB	14115	15255	1.096	1.235	1.166	19090	0.107669	0.0034	0.0316
Ambystoma tigrinum	0	16375	ND1	2698	3669	0.702	0.820	0.761	12461	0.101461	0.008858	0.0873

Ambystoma tigrinum	0	16375	ND2	3884	4927	0.847	0.974	0.910	14906	0.117718	0.001565	0.0133
Ambystoma tigrinum	0	16375	ND3	9458	9805	0.527	0.570	0.549	8983	0.374737	0.000037	0.0001
Ambystoma tigrinum	0	16375	ND4	10163	11537	0.614	0.781	0.697	11420	0.115292	0.000012	0.0001
Ambystoma tigrinum	0	16375	ND4L	9873	10169	0.578	0.614	0.596	9762	0.093728	0.000009	0.0001
Ambystoma tigrinum	0	16375	ND5	11750	13549	0.807	1.027	0.917	15019	0.112761	0.002361	0.0209
Ambystoma tigrinum	0	16375	ND6	13526	14044	1.024	1.088	1.056	17290	0.108524	0.01393	0.1284
Andrias davidianus	0	16503	ATP6	7912	8593	0.336	0.418	0.377	6222	0.126991	0.014206	0.534
Andrias davidianus	0	16503	ATP8	7754	7921	0.317	0.337	0.327	5393	0.063765	0.034049	0.534
Andrias davidianus	0	16503	COX1	5305	6855	0.020	0.208	0.114	1878	0.227497	0.00363	0.016
Andrias davidianus	0	16503	COX2	6997	7684	0.225	0.308	0.267	4399	0.041872	0.004262	0.1018
Andrias davidianus	0	16503	COX3	8595	9378	0.419	0.514	0.466	7691	0.080612	0.008747	0.1085
Andrias davidianus	0	16503	CYTB	14132	15272	1.090	1.228	1.159	19122	0.125139	0.004894	0.0391
Andrias davidianus	0	16503	ND1	2712	3681	0.706	0.823	0.764	12614	0.121821	0.016832	0.1382
Andrias davidianus	0	16503	ND2	3892	4930	0.849	0.974	0.912	15042	0.083773	0.010391	0.124
Andrias davidianus	0	16503	ND3	9450	9800	0.522	0.565	0.543	8968	0.077478	0.012877	0.1662
Andrias davidianus	0	16503	ND4	10158	11532	0.608	0.775	0.691	11408	0.064903	0.002569	0.0396
Andrias davidianus	0	16503	ND4L	9868	10164	0.573	0.609	0.591	9750	0.000005	0	--
Andrias davidianus	0	16503	ND5	11745	13556	0.800	1.020	0.910	15019	0.140753	0.010158	0.0722
Andrias davidianus	0	16503	ND6	13542	14060	1.018	1.081	1.050	17320	0.11371	0.010326	0.0908
Andrias japonicus	0	16298	ATP6	7908	8591	0.340	0.424	0.382	6225	0.092003	0.013698	0.0001
Andrias japonicus	0	16298	ATP8	7750	7917	0.321	0.341	0.331	5393	0.121432	0.000012	0.0001
Andrias japonicus	0	16298	COX1	5301	6851	0.020	0.210	0.115	1878	0.000005	0	--
Andrias japonicus	0	16298	COX2	6993	7680	0.228	0.312	0.270	4400	0.097203	0.008652	0.089
Andrias japonicus	0	16298	COX3	8591	9374	0.424	0.520	0.472	7691	0.090668	0.009621	0.1061
Andrias japonicus	0	16298	CYTB	14124	15264	1.103	1.243	1.173	19113	0.185725	0.011626	0.0626
Andrias japonicus	0	16298	ND1	2707	3678	0.702	0.821	0.761	12409	0.129851	0.018422	0.1419
Andrias japonicus	0	16298	ND2	3887	4927	0.847	0.974	0.910	14838	0.145808	0.015196	0.1042

Andrias japonicus	0	16298	ND3	9445	9795	0.529	0.572	0.550	8966	0.024387	0.003281	0.1345
Andrias japonicus	0	16298	ND4	10153	11529	0.616	0.784	0.700	11408	0.153568	0.009409	0.0613
Andrias japonicus	0	16298	ND4L	9863	10159	0.580	0.616	0.598	9748	0.16446	0.004834	0.0294
Andrias japonicus	0	16298	ND5	11737	13548	0.810	1.032	0.921	15011	0.117176	0.009335	0.0797
Andrias japonicus	0	16298	ND6	13534	14052	1.030	1.094	1.062	17312	0.118038	0.003345	0.0283
Aneides flavipunctatus	1	20197	ATP6	7807	8490	0.274	0.342	0.308	6223	0.987624	0.050902	0.1648
Aneides flavipunctatus	1	20197	ATP8	7652	7817	0.259	0.275	0.267	5395	0.704215	0.116057	0.1648
Aneides flavipunctatus	1	20197	COX1	5205	6752	0.017	0.170	0.093	1882	0.815556	0.009959	0.0122
Aneides flavipunctatus	1	20197	COX2	6893	7580	0.184	0.252	0.218	4399	0.764352	0.012407	0.0162
Aneides flavipunctatus	1	20197	COX3	8490	9273	0.342	0.420	0.381	7689	0.642163	0.029936	0.0466
Aneides flavipunctatus	1	20197	CYTB	13434	14574	0.832	0.944	0.888	17934	0.768453	0.040389	0.0526
Aneides flavipunctatus	1	20197	ND1	2627	3583	0.761	0.856	0.809	16333	0.711567	0.033117	0.0465
Aneides flavipunctatus	1	20197	ND2	3789	4829	0.876	0.979	0.928	18741	0.71605	0.070467	0.0984
Aneides flavipunctatus	1	20197	ND3	9340	9684	0.426	0.460	0.443	8950	1.188128	0.059981	0.0505
Aneides flavipunctatus	1	20197	ND4	10040	11414	0.495	0.632	0.563	11380	0.898974	0.038827	0.0432
Aneides flavipunctatus	1	20197	ND4L	9750	10046	0.467	0.496	0.481	9722	0.477065	0.032259	0.0676
Aneides flavipunctatus	1	20197	ND5	11621	13433	0.652	0.831	0.742	14980	0.797685	0.046358	0.0581
Aneides flavipunctatus	1	20197	ND6	17692	18207	1.253	1.304	1.279	25826	0.903357	0.029495	0.0326
Aneides hardii	1	22184	ATP6	12454	13137	0.250	0.312	0.281	6227	0.578357	0.033263	0.1165
Aneides hardii	1	22184	ATP8	12296	12464	0.236	0.251	0.243	5396	0.4889	0.056939	0.1165
Aneides hardii	1	22184	COX1	9853	11393	0.015	0.154	0.085	1882	0.498958	0.002716	0.0054
Aneides hardii	1	22184	COX2	11534	12221	0.167	0.229	0.198	4391	0.302081	0.009957	0.033
Aneides hardii	1	22184	COX3	13137	13920	0.312	0.382	0.347	7693	0.491697	0.0263	0.0535

Aneides hardii	1	22184	CYTB	18093	19233	0.758	0.861	0.810	17962	0.639894	0.022865	0.0357
Aneides hardii	1	22184	ND1	7265	8224	0.782	0.869	0.825	18310	0.533768	0.01903	0.0357
Aneides hardii	1	22184	ND2	8431	9471	0.887	0.981	0.934	20722	0.531787	0.042396	0.0797
Aneides hardii	1	22184	ND3	13987	14331	0.388	0.419	0.404	8953	0.396857	0.019878	0.0501
Aneides hardii	1	22184	ND4	14687	16061	0.451	0.575	0.513	11384	0.384082	0.023779	0.0619
Aneides hardii	1	22184	ND4L	14397	14693	0.425	0.452	0.438	9727	0.72666	0.031327	0.0431
Aneides hardii	1	22184	ND5	16268	18082	0.594	0.757	0.676	14986	0.474619	0.020599	0.0434
Aneides hardii	1	22184	ND6	4959	5477	0.574	0.621	0.598	13256	0.340753	0.004799	0.0141
Batrachoseps attenuatus	1	17559	ATP6	8074	8757	0.343	0.421	0.382	6706	1.572983	0.054103	0.1516
Batrachoseps attenuatus	1	17559	ATP8	7916	8083	0.325	0.344	0.335	5873	0.634025	0.096142	0.1516
Batrachoseps attenuatus	1	17559	COX1	5465	7012	0.046	0.222	0.134	2351	0.897082	0.008244	0.0092
Batrachoseps attenuatus	1	17559	COX2	7154	7841	0.238	0.316	0.277	4869	0.615318	0.018809	0.0306
Batrachoseps attenuatus	1	17559	COX3	8757	9540	0.421	0.510	0.465	8171	1.192856	0.029669	0.0249
Batrachoseps attenuatus	1	17559	CYTB	14292	15432	1.051	1.181	1.116	19598	0.835582	0.020371	0.0244
Batrachoseps attenuatus	1	17559	ND1	2644	3604	0.725	0.834	0.779	13681	0.841906	0.027057	0.0321
Batrachoseps attenuatus	1	17559	ND2	3820	4860	0.858	0.977	0.918	16113	1.253357	0.065211	0.052
Batrachoseps attenuatus	1	17559	ND3	9607	9952	0.518	0.557	0.537	9434	1.337667	0.040479	0.0303
Batrachoseps attenuatus	1	17559	ND4	10310	11684	0.598	0.754	0.676	11867	1.211108	0.04092	0.0338
Batrachoseps attenuatus	1	17559	ND4L	10020	10316	0.565	0.598	0.581	10210	0.950906	0.047666	0.0501
Batrachoseps attenuatus	1	17559	ND5	11891	13720	0.778	0.986	0.882	15484	1.207669	0.051253	0.0424
Batrachoseps attenuatus	1	17559	ND6	13703	14221	0.984	1.043	1.014	17798	1.3254	0.052167	0.0394
Batrachoseps wrightorum	1	19789	ATP6	7846	8529	0.281	0.350	0.315	6237	1.080725	0.055726	0.1027

Batrachoseps wrightorum	1	19789	ATP8	7688	7855	0.265	0.282	0.273	5405	0.582024	0.059746	0.1027
Batrachoseps wrightorum	1	19789	COX1	5237	6784	0.017	0.173	0.095	1883	0.682972	0.0105	0.0154
Batrachoseps wrightorum	1	19789	COX2	6927	7614	0.188	0.257	0.223	4403	0.742373	0.02299	0.031
Batrachoseps wrightorum	1	19789	COX3	8529	9312	0.350	0.429	0.389	7703	0.9737	0.032099	0.033
Batrachoseps wrightorum	1	19789	CYTB	14056	15196	0.908	1.024	0.966	19114	0.95119	0.042224	0.0444
Batrachoseps wrightorum	1	19789	ND1	2648	3607	0.755	0.852	0.804	15905	1.106118	0.029666	0.0268
Batrachoseps wrightorum	1	19789	ND2	3821	4861	0.874	0.979	0.926	18334	0.923919	0.056151	0.0608
Batrachoseps wrightorum	1	19789	ND3	9378	9725	0.436	0.471	0.453	8965	0.864893	0.024373	0.0282
Batrachoseps wrightorum	1	19789	ND4	10081	11455	0.507	0.645	0.576	11397	0.9995	0.034658	0.0347
Batrachoseps wrightorum	1	19789	ND4L	9791	10087	0.477	0.507	0.492	9740	0.380042	0.009167	0.0241
Batrachoseps wrightorum	1	19789	ND5	11662	13482	0.666	0.850	0.758	15006	0.7393	0.032668	0.0442
Batrachoseps wrightorum	1	19789	ND6	13468	13986	0.849	0.901	0.875	17316	1.031785	0.054289	0.0526
Batrachuperus londongensis	0	16379	ATP6	7955	8638	0.339	0.423	0.381	6240	0.232172	0.02444	0.1792
Batrachuperus londongensis	0	16379	ATP8	7797	7964	0.320	0.340	0.330	5408	0.151384	0.027135	0.1792
Batrachuperus londongensis	0	16379	COX1	5345	6895	0.021	0.210	0.115	1888	0.227435	0.001747	0.0077
Batrachuperus londongensis	0	16379	COX2	7037	7727	0.227	0.312	0.269	4412	0.129283	0.001956	0.0151
Batrachuperus londongensis	0	16379	COX3	8639	9422	0.423	0.519	0.471	7710	0.228085	0.004874	0.0214
Batrachuperus londongensis	0	16379	CYTB	14182	15322	1.100	1.239	1.169	19152	0.217009	0.011454	0.0528
Batrachuperus londongensis	0	16379	ND1	2741	3712	0.703	0.821	0.762	12480	0.16093	0.007528	0.0468

Batrachuperus londongensis	0	16379	ND2	3921	4964	0.847	0.974	0.910	14912	0.182311	0.002724	0.0149
Batrachuperus londongensis	0	16379	ND3	9491	9841	0.527	0.570	0.548	8980	0.202422	0.004728	0.0234
Batrachuperus londongensis	0	16379	ND4	10199	11576	0.613	0.782	0.697	11423	0.156579	0.007386	0.0472
Batrachuperus londongensis	0	16379	ND4L	9909	10205	0.578	0.614	0.596	9762	0.170577	0.009052	0.0531
Batrachuperus londongensis	0	16379	ND5	11786	13606	0.807	1.029	0.918	15040	0.240728	0.01802	0.0749
Batrachuperus londongensis	0	16379	ND6	13592	14110	1.028	1.091	1.059	17350	0.155423	0.000016	0.0001
Batrachuperus pinchonii	0	16390	ATP6	7961	8644	0.339	0.423	0.381	6241	0.200164	0.014895	0.0001
Batrachuperus pinchonii	0	16390	ATP8	7803	7970	0.320	0.340	0.330	5409	0.063439	0.000006	0.0001
Batrachuperus pinchonii	0	16390	COX1	5353	6903	0.021	0.210	0.115	1892	0.203146	0.001737	0.0085
Batrachuperus pinchonii	0	16390	COX2	7046	7730	0.228	0.311	0.269	4412	0.213654	0.000021	0.0001
Batrachuperus pinchonii	0	16390	COX3	8644	9428	0.423	0.518	0.470	7708	0.133922	0.003331	0.0249
Batrachuperus pinchonii	0	16390	CYTB	14192	15333	1.100	1.239	1.169	19162	0.210275	0.012028	0.0572
Batrachuperus pinchonii	0	16390	ND1	2746	3717	0.703	0.821	0.762	12488	0.107982	0.00661	0.0612
Batrachuperus pinchonii	0	16390	ND2	3926	4969	0.847	0.974	0.910	14921	0.1567	0.009764	0.0623
Batrachuperus pinchonii	0	16390	ND3	9498	9848	0.527	0.569	0.548	8983	0.167293	0.004699	0.0281
Batrachuperus pinchonii	0	16390	ND4	10206	11583	0.613	0.781	0.697	11425	0.227848	0.011627	0.051
Batrachuperus pinchonii	0	16390	ND4L	9916	10212	0.578	0.614	0.596	9764	0.137518	0.004474	0.0325
Batrachuperus pinchonii	0	16390	ND5	11793	13616	0.807	1.029	0.918	15045	0.143352	0.007355	0.0513
Batrachuperus pinchonii	0	16390	ND6	13602	14120	1.028	1.091	1.059	17359	0.263568	0.013477	0.0511

Batrachuperus tibetanus	0	16379	ATP6	7953	8636	0.339	0.423	0.381	6239	0.175815	0.009874	0.0001
Batrachuperus tibetanus	0	16379	ATP8	7795	7962	0.320	0.340	0.330	5407	0.0431	0.000004	0.0001
Batrachuperus tibetanus	0	16379	COX1	5345	6895	0.021	0.210	0.115	1890	0.187105	0.001737	0.0093
Batrachuperus tibetanus	0	16379	COX2	7038	7722	0.228	0.311	0.269	4410	0.115349	0.00195	0.0169
Batrachuperus tibetanus	0	16379	COX3	8636	9420	0.423	0.518	0.470	7706	0.117235	0.000012	0.0001
Batrachuperus tibetanus	0	16379	CYTB	14180	15321	1.100	1.239	1.169	19151	0.224604	0.005716	0.0254
Batrachuperus tibetanus	0	16379	ND1	2737	3708	0.702	0.821	0.762	12474	0.235392	0.021406	0.0909
Batrachuperus tibetanus	0	16379	ND2	3918	4961	0.847	0.974	0.910	14908	0.155171	0.008169	0.0526
Batrachuperus tibetanus	0	16379	ND3	9489	9839	0.527	0.570	0.548	8978	0.197145	0.00002	0.0001
Batrachuperus tibetanus	0	16379	ND4	10197	11571	0.613	0.781	0.697	11418	0.185403	0.004602	0.0248
Batrachuperus tibetanus	0	16379	ND4L	9907	10203	0.578	0.614	0.596	9760	0.094137	0.004527	0.0481
Batrachuperus tibetanus	0	16379	ND5	11780	13603	0.807	1.029	0.918	15033	0.164713	0.008735	0.053
Batrachuperus tibetanus	0	16379	ND6	13589	14107	1.027	1.091	1.059	17346	0.168369	0.013176	0.0783
Batrachuperus yenyuanensis	0	16394	ATP6	7964	8647	0.339	0.422	0.381	6240	0.252902	0.010337	0.0001
Batrachuperus yenyuanensis	0	16394	ATP8	7806	7973	0.320	0.340	0.330	5409	0.164214	0.000016	0.0001
Batrachuperus yenyuanensis	0	16394	COX1	5353	6903	0.021	0.210	0.115	1886	0.305768	0.005258	0.0172
Batrachuperus yenyuanensis	0	16394	COX2	7046	7733	0.227	0.311	0.269	4408	0.170448	0.001918	0.0113
Batrachuperus yenyuanensis	0	16394	COX3	8647	9430	0.422	0.518	0.470	7707	0.265492	0.010668	0.0402
Batrachuperus yenyuanensis	0	16394	CYTB	14194	15334	1.099	1.238	1.169	19158	0.280036	0.017414	0.0622

Batrachuperus yenyuanensis	0	16394	ND1	2747	3716	0.703	0.821	0.762	12487	0.179768	0.00749	0.0417
Batrachuperus yenyuanensis	0	16394	ND2	3928	4969	0.847	0.974	0.910	14921	0.198135	0.011708	0.0591
Batrachuperus yenyuanensis	0	16394	ND3	9500	9850	0.526	0.569	0.548	8980	0.319845	0.015265	0.0477
Batrachuperus yenyuanensis	0	16394	ND4	10208	11578	0.613	0.780	0.696	11416	0.262217	0.019775	0.0754
Batrachuperus yenyuanensis	0	16394	ND4L	9918	10214	0.577	0.614	0.595	9762	0.039411	0.00509	0.1292
Batrachuperus yenyuanensis	0	16394	ND5	11796	13616	0.807	1.029	0.918	15041	0.280503	0.01787	0.0637
Batrachuperus yenyuanensis	0	16394	ND6	13602	14120	1.027	1.090	1.058	17351	0.249287	0.018219	0.0731
Bolitoglossa sp	1	21657	ATP6	7797	8480	0.255	0.319	0.287	6214	2.31764	0.149201	0.1097
Bolitoglossa sp	1	21657	ATP8	7645	7806	0.241	0.256	0.249	5389	0.382017	0.041892	0.1097
Bolitoglossa sp	1	21657	COX1	5202	6752	0.016	0.159	0.087	1892	1.464709	0.017964	0.0123
Bolitoglossa sp	1	21657	COX2	6887	7574	0.171	0.235	0.203	4399	1.38067	0.026824	0.0194
Bolitoglossa sp	1	21657	COX3	8480	9263	0.319	0.391	0.355	7681	1.314383	0.0475	0.0361
Bolitoglossa sp	1	21657	CYTB	13994	15134	0.828	0.933	0.880	19066	1.887748	0.067598	0.0358
Bolitoglossa sp	1	21657	ND1	2610	3581	0.776	0.866	0.821	17786	1.845182	0.071332	0.0387
Bolitoglossa sp	1	21657	ND2	3793	4824	0.886	0.981	0.933	20212	1.981615	0.120158	0.0606
Bolitoglossa sp	1	21657	ND3	9329	9676	0.397	0.429	0.413	8943	1.0993	0.03811	0.0347
Bolitoglossa sp	1	21657	ND4	10032	11406	0.462	0.589	0.525	11375	1.596559	0.072247	0.0453
Bolitoglossa sp	1	21657	ND4L	9742	10038	0.435	0.462	0.449	9719	1.993988	0.138882	0.0697
Bolitoglossa sp	1	21657	ND5	11608	13408	0.607	0.774	0.691	14954	1.527952	0.089614	0.0586
Bolitoglossa sp	1	21657	ND6	13408	13926	0.774	0.821	0.798	17271	1.797665	0.093332	0.0519
Desmognathus fuscus	0	16628	ATP6	7850	8533	0.334	0.416	0.375	6231	0.798437	0.062586	0.0938
Desmognathus fuscus	0	16628	ATP8	7692	7859	0.315	0.335	0.325	5398	0.644536	0.060436	0.0938
Desmognathus fuscus	0	16628	COX1	5245	6798	0.020	0.207	0.114	1891	0.712621	0.004314	0.0061
Desmognathus fuscus	0	16628	COX2	6931	7618	0.223	0.306	0.264	4396	0.828751	0.025054	0.0302

Desmognathus fuscus	0	16628	COX3	8533	9316	0.416	0.510	0.463	7697	0.69779	0.017746	0.0254
Desmognathus fuscus	0	16628	CYTB	14044	15184	1.079	1.216	1.147	19076	0.80865	0.042165	0.0521
Desmognathus fuscus	0	16628	ND1	2664	3629	0.710	0.826	0.768	12769	0.707032	0.021198	0.03
Desmognathus fuscus	0	16628	ND2	3838	4869	0.851	0.975	0.913	15183	0.667931	0.035433	0.053
Desmognathus fuscus	0	16628	ND3	9383	9727	0.518	0.559	0.539	8958	0.403191	0.036105	0.0895
Desmognathus fuscus	0	16628	ND4	10082	11453	0.602	0.767	0.685	11383	0.608864	0.016278	0.0267
Desmognathus fuscus	0	16628	ND4L	9792	10088	0.567	0.603	0.585	9727	0.857742	0.014506	0.0169
Desmognathus fuscus	0	16628	ND5	11660	13459	0.792	1.008	0.900	14967	0.575638	0.037039	0.0643
Desmognathus fuscus	0	16628	ND6	13455	13973	1.008	1.070	1.039	17276	0.495347	0.041982	0.0848
Desmognathus wrighti	0	16578	ATP6	7918	8601	0.345	0.428	0.387	6407	0.969838	0.039893	0.252
Desmognathus wrighti	0	16578	ATP8	7763	7927	0.327	0.346	0.337	5578	0.466593	0.117598	0.252
Desmognathus wrighti	0	16578	COX1	5224	6775	0.020	0.207	0.114	1887	0.729728	0.014564	0.02
Desmognathus wrighti	0	16578	COX2	7004	7688	0.235	0.318	0.276	4580	0.944641	0.047946	0.0508
Desmognathus wrighti	0	16578	COX3	8601	9384	0.428	0.522	0.475	7873	0.947972	0.033203	0.035
Desmognathus wrighti	0	16578	CYTB	14096	15236	1.091	1.228	1.159	19220	0.734527	0.030241	0.0412
Desmognathus wrighti	0	16578	ND1	2638	3598	0.708	0.824	0.766	12702	1.673526	0.072415	0.0433
Desmognathus wrighti	0	16578	ND2	3808	4840	0.849	0.974	0.912	15113	0.671738	0.050411	0.075
Desmognathus wrighti	0	16578	ND3	9452	9796	0.530	0.572	0.551	9135	1.964325	0.086615	0.0441
Desmognathus wrighti	0	16578	ND4	10153	11518	0.615	0.780	0.697	11559	1.047242	0.072158	0.0689

Desmognathus wrighti	0	16578	ND4L	9863	10159	0.580	0.616	0.598	9909	1.4376	0.089249	0.0621
Desmognathus wrighti	0	16578	ND5	11724	13523	0.804	1.022	0.913	15135	0.882106	0.056031	0.0635
Desmognathus wrighti	0	16578	ND6	13520	14026	1.021	1.082	1.052	17434	0.690214	0.073744	0.1068
Echinotriton andersoni	0	16268	ATP6	7923	8606	0.342	0.426	0.384	6245	0.372984	0.034352	0.0725
Echinotriton andersoni	0	16268	ATP8	7765	7932	0.323	0.343	0.333	5413	0.368571	0.026709	0.0725
Echinotriton andersoni	0	16268	COX1	5310	6869	0.021	0.212	0.117	1895	0.468133	0.006797	0.0145
Echinotriton andersoni	0	16268	COX2	7003	7690	0.229	0.313	0.271	4409	0.514541	0.024607	0.0478
Echinotriton andersoni	0	16268	COX3	8606	9389	0.426	0.522	0.474	7711	0.671533	0.027904	0.0416
Echinotriton andersoni	0	16268	CYTB	14132	15272	1.105	1.245	1.175	19120	0.620269	0.030468	0.0491
Echinotriton andersoni	0	16268	ND1	2705	3673	0.700	0.819	0.760	12362	0.564627	0.025884	0.0458
Echinotriton andersoni	0	16268	ND2	3888	4931	0.846	0.974	0.910	14803	0.467265	0.029159	0.0624
Echinotriton andersoni	0	16268	ND3	9458	9805	0.531	0.573	0.552	8979	0.553089	0.035511	0.0642
Echinotriton andersoni	0	16268	ND4	10162	11539	0.617	0.787	0.702	11418	0.488556	0.035434	0.0725
Echinotriton andersoni	0	16268	ND4L	9872	10168	0.582	0.618	0.600	9756	0.571353	0.039134	0.0685
Echinotriton andersoni	0	16268	ND5	11747	13558	0.812	1.035	0.923	15021	0.361074	0.020199	0.0559
Echinotriton andersoni	0	16268	ND6	13544	14062	1.033	1.097	1.065	17321	0.393416	0.017672	0.0449
Ensatina eschscholtzii	1	22816	ATP6	7835	8518	0.244	0.304	0.274	6247	1.564734	0.101388	0.281
Ensatina eschscholtzii	1	22816	ATP8	7683	7841	0.231	0.244	0.237	5418	1.207647	0.33936	0.281
Ensatina eschscholtzii	1	22816	COX1	5228	6787	0.015	0.152	0.084	1909	0.937859	0.030849	0.0329

<i>Ensatina eschscholtzii</i>	1	22816	COX2	6913	7593	0.163	0.223	0.193	4400	2.563301	0.110675	0.0432
<i>Ensatina eschscholtzii</i>	1	22816	COX3	8518	9301	0.304	0.372	0.338	7713	1.129632	0.049686	0.044
<i>Ensatina eschscholtzii</i>	1	22816	CYTB	14021	15161	0.786	0.886	0.836	19075	1.285902	0.043233	0.0336
<i>Ensatina eschscholtzii</i>	1	22816	ND1	2632	3594	0.788	0.872	0.830	18936	1.197988	0.052997	0.0442
<i>Ensatina eschscholtzii</i>	1	22816	ND2	3801	4841	0.890	0.981	0.936	21352	1.09703	0.066187	0.0603
<i>Ensatina eschscholtzii</i>	1	22816	ND3	9370	9714	0.378	0.409	0.394	8978	0.717345	0.047042	0.0656
<i>Ensatina eschscholtzii</i>	1	22816	ND4	10069	11440	0.440	0.560	0.500	11403	1.049482	0.055808	0.0532
<i>Ensatina eschscholtzii</i>	1	22816	ND4L	9779	10075	0.414	0.440	0.427	9748	1.025525	0.057093	0.0557
<i>Ensatina eschscholtzii</i>	1	22816	ND5	11660	13459	0.579	0.737	0.658	15014	1.409584	0.090201	0.064
<i>Ensatina eschscholtzii</i>	1	22816	ND6	13435	13950	0.735	0.780	0.757	17279	0.946867	0.09433	0.0996
<i>Eurycea bislineata</i>	0	17184	ATP6	7865	8548	0.322	0.401	0.362	6215	1.364233	0.065532	0.1366
<i>Eurycea bislineata</i>	0	17184	ATP8	7710	7874	0.304	0.323	0.313	5386	0.50181	0.06857	0.1366
<i>Eurycea bislineata</i>	0	17184	COX1	5265	6821	0.019	0.200	0.110	1888	0.798026	0.00259	0.0032
<i>Eurycea bislineata</i>	0	17184	COX2	6948	7635	0.215	0.295	0.255	4385	0.680158	0.014258	0.021
<i>Eurycea bislineata</i>	0	17184	COX3	8548	9328	0.401	0.492	0.447	7678	0.830845	0.020675	0.0249
<i>Eurycea bislineata</i>	0	17184	CYTB	14059	15199	1.043	1.176	1.109	19060	0.825742	0.027408	0.0332
<i>Eurycea bislineata</i>	0	17184	ND1	2660	3625	0.716	0.828	0.772	13270	1.01071	0.032324	0.032
<i>Eurycea bislineata</i>	0	17184	ND2	3840	4880	0.854	0.975	0.914	15706	1.264661	0.072982	0.0577
<i>Eurycea bislineata</i>	0	17184	ND3	9401	9743	0.501	0.541	0.521	8946	0.791401	0.018053	0.0228
<i>Eurycea bislineata</i>	0	17184	ND4	10102	11469	0.582	0.741	0.662	11373	1.016326	0.042975	0.0423
<i>Eurycea bislineata</i>	0	17184	ND4L	9812	10108	0.549	0.583	0.566	9722	0.949369	0.061967	0.0653
<i>Eurycea bislineata</i>	0	17184	ND5	11676	13484	0.766	0.976	0.871	14962	1.277164	0.051798	0.0406
<i>Eurycea bislineata</i>	0	17184	ND6	13470	13988	0.974	1.035	1.004	17260	0.507657	0.019748	0.0389
<i>Gyrinophilus porphyriticus</i>	0	16778	ATP6	7863	8546	0.330	0.412	0.371	6223	0.49832	0.036104	0.4369

Gyrinophilus porphyriticus	0	16778	ATP8	7708	7872	0.312	0.331	0.322	5394	0.18677	0.081603	0.4369
Gyrinophilus porphyriticus	0	16778	COX1	5261	6805	0.020	0.204	0.112	1880	0.392834	0.000039	0.0001
Gyrinophilus porphyriticus	0	16778	COX2	6947	7634	0.221	0.303	0.262	4395	0.367562	0.003627	0.0099
Gyrinophilus porphyriticus	0	16778	COX3	8546	9329	0.412	0.505	0.458	7689	0.334522	0.002177	0.0065
Gyrinophilus porphyriticus	0	16778	CYTB	14064	15204	1.069	1.205	1.137	19082	0.637214	0.027118	0.0426
Gyrinophilus porphyriticus	0	16778	ND1	2672	3631	0.711	0.826	0.769	12895	0.409179	0.017452	0.0427
Gyrinophilus porphyriticus	0	16778	ND2	3845	4885	0.851	0.975	0.913	15322	0.487785	0.036315	0.0744
Gyrinophilus porphyriticus	0	16778	ND3	9398	9745	0.513	0.555	0.534	8957	0.451403	0.01192	0.0264
Gyrinophilus porphyriticus	0	16778	ND4	10103	11474	0.597	0.761	0.679	11391	0.509117	0.020707	0.0407
Gyrinophilus porphyriticus	0	16778	ND4L	9813	10109	0.563	0.598	0.580	9735	0.115283	0.008924	0.0774
Gyrinophilus porphyriticus	0	16778	ND5	11681	13476	0.785	0.999	0.892	14971	0.42874	0.019092	0.0445
Gyrinophilus porphyriticus	0	16778	ND6	13478	13996	1.000	1.061	1.030	17288	0.345734	0.004321	0.0125
Hemidactylum scutatum	0	17421	ATP6	7807	8487	0.316	0.395	0.355	6192	2.065957	0.087491	0.1684
Hemidactylum scutatum	0	17421	ATP8	7655	7816	0.299	0.317	0.308	5368	1.039288	0.175025	0.1684
Hemidactylum scutatum	0	17421	COX1	5217	6764	0.019	0.197	0.108	1880	1.29605	0.01363	0.0105
Hemidactylum scutatum	0	17421	COX2	6896	7583	0.212	0.291	0.251	4377	0.985342	0.01871	0.019
Hemidactylum scutatum	0	17421	COX3	8487	9270	0.395	0.484	0.439	7656	1.247898	0.03486	0.0279
Hemidactylum scutatum	0	17421	CYTB	13997	15137	1.027	1.158	1.092	19032	1.259411	0.04386	0.0348
Hemidactylum scutatum	0	17421	ND1	2622	3590	0.721	0.832	0.777	13531	1.768121	0.054606	0.0309

Hemidactylum scutatum	0	17421	ND2	3800	4840	0.856	0.976	0.916	15959	1.773023	0.097683	0.0551
Hemidactylum scutatum	0	17421	ND3	9337	9679	0.492	0.531	0.512	8913	2.457594	0.107422	0.0437
Hemidactylum scutatum	0	17421	ND4	10038	11403	0.573	0.729	0.651	11338	1.567208	0.064432	0.0411
Hemidactylum scutatum	0	17421	ND4L	9748	10044	0.539	0.573	0.556	9690	1.650937	0.066498	0.0403
Hemidactylum scutatum	0	17421	ND5	11609	13412	0.753	0.960	0.856	14919	1.350316	0.064633	0.0479
Hemidactylum scutatum	0	17421	ND6	13412	13927	0.960	1.019	0.989	17237	1.619326	0.091667	0.0566
Hydromantes brunus	1	17220	ATP6	8301	8984	0.318	0.398	0.358	6166	0.998537	0.058404	0.2345
Hydromantes brunus	1	17220	ATP8	8143	8310	0.300	0.319	0.310	5333	0.337943	0.079245	0.2345
Hydromantes brunus	1	17220	COX1	5695	7239	0.016	0.195	0.105	1814	0.839676	0.009765	0.0116
Hydromantes brunus	1	17220	COX2	7385	8072	0.212	0.292	0.252	4338	0.708366	0.02957	0.0417
Hydromantes brunus	1	17220	COX3	8984	9767	0.398	0.489	0.443	7631	0.856177	0.028157	0.0329
Hydromantes brunus	1	17220	CYTB	14497	15637	1.038	1.170	1.104	19014	1.139469	0.047245	0.0415
Hydromantes brunus	1	17220	ND1	2670	3633	0.664	0.776	0.720	12403	1.160136	0.039896	0.0344
Hydromantes brunus	1	17220	ND2	3843	4875	0.801	0.920	0.861	14818	0.945365	0.074513	0.0788
Hydromantes brunus	1	17220	ND3	9836	10183	0.497	0.537	0.517	8898	1.200042	0.066857	0.0557
Hydromantes brunus	1	17220	ND4	10539	11910	0.578	0.738	0.658	11329	0.844286	0.043622	0.0517
Hydromantes brunus	1	17220	ND4L	10249	10545	0.545	0.579	0.562	9674	1.422693	0.068043	0.0478
Hydromantes brunus	1	17220	ND5	12117	13925	0.762	0.972	0.867	14922	0.962797	0.043681	0.0454
Hydromantes brunus	1	17220	ND6	13911	14426	0.970	1.030	1.000	17217	1.650654	0.072922	0.0442
Hynobius amjiensis	0	16401	ATP6	7959	8642	0.339	0.422	0.381	6243	0.280014	0.016614	--
Hynobius amjiensis	0	16401	ATP8	7801	7968	0.320	0.340	0.330	5411	0.000062	0.062333	--
Hynobius amjiensis	0	16401	COX1	5347	6897	0.021	0.210	0.115	1886	0.359145	0.000879	0.0024
Hynobius amjiensis	0	16401	COX2	7042	7728	0.227	0.311	0.269	4412	0.147319	0.000015	0.0001
Hynobius amjiensis	0	16401	COX3	8642	9426	0.422	0.518	0.470	7710	0.248373	0.008491	0.0342
Hynobius amjiensis	0	16401	CYTB	14190	15331	1.099	1.238	1.168	19163	0.309617	0.006234	0.0201
Hynobius amjiensis	0	16401	ND1	2740	3711	0.703	0.821	0.762	12494	0.288807	0.006297	0.0218
Hynobius amjiensis	0	16401	ND2	3922	4965	0.847	0.974	0.910	14930	0.247827	0.011602	0.0468

Hynobius amjiensis	0	16401	ND3	9494	9844	0.526	0.569	0.548	8980	0.471559	0.01551	0.0329
Hynobius amjiensis	0	16401	ND4	10202	11579	0.613	0.780	0.696	11422	0.350294	0.014369	0.041
Hynobius amjiensis	0	16401	ND4L	9912	10208	0.577	0.613	0.595	9763	0.092073	0.009116	0.099
Hynobius amjiensis	0	16401	ND5	11790	13603	0.806	1.027	0.917	15036	0.318304	0.012304	0.0387
Hynobius amjiensis	0	16401	ND6	13599	14117	1.027	1.090	1.058	17358	0.205254	0.000021	0.0001
Hynobius arisanensis	0	16401	ATP6	7964	8647	0.339	0.422	0.381	6245	0.02091	0.008872	--
Hynobius arisanensis	0	16401	ATP8	7806	7973	0.320	0.340	0.330	5413	0.000005	0	--
Hynobius arisanensis	0	16401	COX1	5351	6901	0.021	0.210	0.115	1886	0.011491	0.000933	0.0812
Hynobius arisanensis	0	16401	COX2	7048	7735	0.227	0.311	0.269	4417	0.00867	0.004327	0.499
Hynobius arisanensis	0	16401	COX3	8647	9431	0.422	0.518	0.470	7712	0.009911	0.000001	0.0001
Hynobius arisanensis	0	16401	CYTB	14192	15332	1.099	1.238	1.168	19158	0.020706	0.000953	0.046
Hynobius arisanensis	0	16401	ND1	2746	3717	0.703	0.821	0.762	12498	0.000006	0	--
Hynobius arisanensis	0	16401	ND2	3927	4968	0.847	0.974	0.910	14930	0.031501	0.000003	0.0001
Hynobius arisanensis	0	16401	ND3	9499	9849	0.526	0.569	0.548	8982	0.000005	0	--
Hynobius arisanensis	0	16401	ND4	10207	11584	0.613	0.781	0.697	11425	0.013822	0.002427	0.1756
Hynobius arisanensis	0	16401	ND4L	9917	10213	0.577	0.613	0.595	9764	0.02747	0.000003	0.0001
Hynobius arisanensis	0	16401	ND5	11795	13615	0.806	1.028	0.917	15044	0.003218	0.003836	1.1919
Hynobius arisanensis	0	16401	ND6	13601	14119	1.027	1.090	1.058	17354	0.037643	0.000004	0.0001
Hynobius chinensis	0	16408	ATP6	7977	8660	0.339	0.422	0.381	6247	0	0.000002	--
Hynobius chinensis	0	16408	ATP8	7819	7986	0.320	0.340	0.330	5415	0.000017	0.016502	--
Hynobius chinensis	0	16408	COX1	5364	6914	0.021	0.210	0.115	1888	0.005714	0.000913	0.1598
Hynobius chinensis	0	16408	COX2	7059	7746	0.227	0.311	0.269	4415	0.000006	0	--
Hynobius chinensis	0	16408	COX3	8660	9444	0.422	0.518	0.470	7714	0.000002	0.001898	--

Hynobius chinensis	0	16408	CYTB	14210	15351	1.099	1.238	1.168	19171	0	0.000002	--
Hynobius chinensis	0	16408	ND1	2756	3727	0.703	0.821	0.762	12501	0.009741	0.0015	0.154
Hynobius chinensis	0	16408	ND2	3938	4981	0.847	0.974	0.910	14937	0.000001	0.001447	--
Hynobius chinensis	0	16408	ND3	9512	9862	0.526	0.569	0.548	8984	0.000005	0	--
Hynobius chinensis	0	16408	ND4	10220	11597	0.613	0.780	0.696	11427	0	0.000002	--
Hynobius chinensis	0	16408	ND4L	9930	10226	0.577	0.613	0.595	9766	0.000005	0	--
Hynobius chinensis	0	16408	ND5	11807	13633	0.806	1.029	0.917	15049	0.000002	0.000001	0.4283
Hynobius chinensis	0	16408	ND6	13619	14137	1.027	1.090	1.058	17366	0.000009	0.008503	--
Hynobius formosanus	0	16394	ATP6	7963	8646	0.339	0.422	0.381	6239	0.048216	0.016215	--
Hynobius formosanus	0	16394	ATP8	7805	7972	0.320	0.340	0.330	5407	0.000005	0	--
Hynobius formosanus	0	16394	COX1	5352	6902	0.020	0.210	0.115	1884	0.018521	0.000002	0.0001
Hynobius formosanus	0	16394	COX2	7047	7734	0.227	0.311	0.269	4412	0.000005	0.004732	--
Hynobius formosanus	0	16394	COX3	8646	9430	0.422	0.518	0.470	7706	0.063647	0.001653	0.026
Hynobius formosanus	0	16394	CYTB	14191	15331	1.099	1.238	1.168	19152	0.012651	0.005997	0.4741
Hynobius formosanus	0	16394	ND1	2748	3719	0.703	0.821	0.762	12491	0.027519	0.002876	0.1045
Hynobius formosanus	0	16394	ND2	3929	4970	0.847	0.974	0.910	14923	0.015008	0.000002	0.0001
Hynobius formosanus	0	16394	ND3	9498	9848	0.526	0.569	0.548	8977	0.028091	0.005019	0.1787
Hynobius formosanus	0	16394	ND4	10206	11583	0.613	0.781	0.697	11418	0.014687	0.000001	0.0001
Hynobius formosanus	0	16394	ND4L	9916	10212	0.577	0.613	0.595	9759	0.000005	0	--
Hynobius formosanus	0	16394	ND5	11794	13614	0.806	1.028	0.917	15038	0.010694	0.000753	0.0704
Hynobius formosanus	0	16394	ND6	13600	14118	1.027	1.090	1.058	17348	0.000005	0	--
Hynobius guabangshanensis	0	16408	ATP6	7977	8660	0.335	0.419	0.377	6183	0	0.000002	--

<i>Hynobius guabangshanensis</i>	0	16408	ATP8	7819	7986	0.316	0.336	0.326	5351	0.000005	0	--
<i>Hynobius guabangshanensis</i>	0	16408	COX1	5364	6914	0.017	0.206	0.111	1824	0.000005	0	--
<i>Hynobius guabangshanensis</i>	0	16408	COX2	7059	7746	0.223	0.307	0.265	4351	0.000006	0	--
<i>Hynobius guabangshanensis</i>	0	16408	COX3	8660	9443	0.419	0.514	0.466	7649	0.000005	0	--
<i>Hynobius guabangshanensis</i>	0	16408	CYTB	14210	15351	1.095	1.234	1.165	19107	0	0.000002	--
<i>Hynobius guabangshanensis</i>	0	16408	ND1	2756	3726	0.699	0.817	0.758	12436	0.000006	0	--
<i>Hynobius guabangshanensis</i>	0	16408	ND2	3938	4979	0.843	0.970	0.906	14871	0.000002	0.000001	0.4341
<i>Hynobius guabangshanensis</i>	0	16408	ND3	9512	9862	0.522	0.565	0.544	8920	0.000005	0	--
<i>Hynobius guabangshanensis</i>	0	16408	ND4	10220	11597	0.609	0.777	0.693	11363	0	0.000002	--
<i>Hynobius guabangshanensis</i>	0	16408	ND4L	9930	10226	0.573	0.609	0.591	9702	0.000005	0	--
<i>Hynobius guabangshanensis</i>	0	16408	ND5	11807	13633	0.802	1.025	0.913	14985	0.000002	0.000001	0.4281
<i>Hynobius guabangshanensis</i>	0	16408	ND6	13619	14137	1.023	1.086	1.055	17302	0	0.000002	--
<i>Hynobius leechii</i>	0	16428	ATP6	7969	8652	0.339	0.422	0.380	6245	0.012707	0.003372	--
<i>Hynobius leechii</i>	0	16428	ATP8	7811	7978	0.319	0.340	0.330	5413	0.000005	0	--
<i>Hynobius leechii</i>	0	16428	COX1	5356	6906	0.021	0.209	0.115	1887	0.023504	0.002949	0.1255
<i>Hynobius leechii</i>	0	16428	COX2	7051	7737	0.227	0.310	0.269	4412	0.017441	0.002212	0.1268
<i>Hynobius leechii</i>	0	16428	COX3	8652	9436	0.422	0.517	0.469	7712	0.061872	0.00165	0.0267
<i>Hynobius leechii</i>	0	16428	CYTB	14199	15340	1.097	1.236	1.166	19162	0.04996	0.00241	0.0482
<i>Hynobius leechii</i>	0	16428	ND1	2749	3720	0.703	0.821	0.762	12521	0.047657	0.000005	0.0001
<i>Hynobius leechii</i>	0	16428	ND2	3931	4974	0.847	0.974	0.910	14957	0.052103	0.006063	0.1164
<i>Hynobius leechii</i>	0	16428	ND3	9504	9854	0.525	0.568	0.547	8982	0.03259	0.000003	0.0001
<i>Hynobius leechii</i>	0	16428	ND4	10212	11589	0.612	0.779	0.695	11425	0.046968	0.000005	0.0001
<i>Hynobius leechii</i>	0	16428	ND4L	9922	10218	0.576	0.612	0.594	9764	0.025022	0.010431	0.4169
<i>Hynobius leechii</i>	0	16428	ND5	11800	13613	0.805	1.026	0.915	15037	0.018337	0.001676	0.0914

Hynobius leechii	0	16428	ND6	13609	14127	1.025	1.088	1.057	17360	0.000005	0	--
Hynobius quelpaertensis	0	16407	ATP6	7967	8650	0.342	0.425	0.384	6293	0.18276	0.0048	0.2556
Hynobius quelpaertensis	0	16407	ATP8	7809	7976	0.323	0.343	0.333	5461	0.115174	0.029435	0.2556
Hynobius quelpaertensis	0	16407	COX1	5355	6905	0.024	0.213	0.118	1936	0.274943	0.001761	0.0064
Hynobius quelpaertensis	0	16407	COX2	7049	7736	0.230	0.314	0.272	4461	0.229691	0.001903	0.0083
Hynobius quelpaertensis	0	16407	COX3	8650	9434	0.425	0.521	0.473	7761	0.20222	0.001584	0.0078
Hynobius quelpaertensis	0	16407	CYTB	14197	15338	1.101	1.240	1.171	19211	0.29338	0.00114	0.0039
Hynobius quelpaertensis	0	16407	ND1	2748	3719	0.706	0.824	0.765	12550	0.283215	0.015814	0.0558
Hynobius quelpaertensis	0	16407	ND2	3930	4971	0.850	0.977	0.913	14984	0.256956	0.008032	0.0313
Hynobius quelpaertensis	0	16407	ND3	9503	9851	0.529	0.572	0.550	9030	0.17884	0.012437	0.0695
Hynobius quelpaertensis	0	16407	ND4	10211	11588	0.616	0.783	0.699	11475	0.360562	0.0096	0.0266
Hynobius quelpaertensis	0	16407	ND4L	9921	10217	0.580	0.616	0.598	9814	0.220478	0.014022	0.0636
Hynobius quelpaertensis	0	16407	ND5	11798	13611	0.809	1.030	0.919	15085	0.312992	0.016407	0.0524
Hynobius quelpaertensis	0	16407	ND6	13607	14125	1.029	1.093	1.061	17408	0.224373	0.004314	0.0192
Hynobius yangi	0	16424	ATP6	7969	8652	0.339	0.422	0.380	6245	0.02376	0.004888	--
Hynobius yangi	0	16424	ATP8	7811	7978	0.319	0.340	0.330	5413	0.000005	0	--
Hynobius yangi	0	16424	COX1	5357	6907	0.021	0.209	0.115	1888	0.033895	0.000003	0.0001
Hynobius yangi	0	16424	COX2	7051	7737	0.227	0.310	0.269	4412	0.021901	0.000002	0.0001
Hynobius yangi	0	16424	COX3	8652	9435	0.422	0.517	0.470	7711	0.010998	0.000001	0.0001
Hynobius yangi	0	16424	CYTB	14197	15337	1.097	1.236	1.167	19159	0.049616	0.003708	0.0747
Hynobius yangi	0	16424	ND1	2749	3719	0.703	0.821	0.762	12516	0.051432	0.000005	0.0001
Hynobius yangi	0	16424	ND2	3931	4972	0.847	0.974	0.910	14951	0.035665	0.005583	0.1565
Hynobius yangi	0	16424	ND3	9504	9852	0.526	0.568	0.547	8980	0.050884	0.011418	0.2244

Hynobius yangi	0	16424	ND4	10212	11589	0.612	0.780	0.696	11425	0.067767	0.005837	0.0861
Hynobius yangi	0	16424	ND4L	9922	10218	0.577	0.613	0.595	9764	0.049966	0.009131	0.1827
Hynobius yangi	0	16424	ND5	11798	13611	0.805	1.026	0.915	15033	0.021775	0.004312	0.198
Hynobius yangi	0	16424	ND6	13607	14125	1.025	1.088	1.057	17356	0.024561	0.000002	0.0001
Liua shihi	0	16376	ATP6	7961	8644	0.339	0.423	0.381	6239	0.265363	0.010641	0.0956
Liua shihi	0	16376	ATP8	7803	7970	0.320	0.340	0.330	5407	0.140513	0.013429	0.0956
Liua shihi	0	16376	COX1	5352	6893	0.021	0.209	0.115	1878	0.266217	0.001009	0.0038
Liua shihi	0	16376	COX2	7043	7730	0.227	0.311	0.269	4408	0.177742	0.003996	0.0225
Liua shihi	0	16376	COX3	8644	9428	0.423	0.518	0.471	7706	0.249292	0.004863	0.0195
Liua shihi	0	16376	CYTB	14180	15321	1.099	1.238	1.169	19135	0.396877	0.01429	0.036
Liua shihi	0	16376	ND1	2747	3718	0.703	0.821	0.762	12475	0.341098	0.012212	0.0358
Liua shihi	0	16376	ND2	3928	4971	0.847	0.974	0.910	14909	0.267568	0.019606	0.0733
Liua shihi	0	16376	ND3	9497	9847	0.527	0.570	0.548	8978	0.284045	0.004788	0.0169
Liua shihi	0	16376	ND4	10205	11579	0.613	0.781	0.697	11417	0.264545	0.003227	0.0122
Liua shihi	0	16376	ND4L	9915	10211	0.578	0.614	0.596	9760	0.146578	0.000015	0.0001
Liua shihi	0	16376	ND5	11789	13603	0.807	1.028	0.918	15026	0.323679	0.016054	0.0496
Liua shihi	0	16376	ND6	13589	14107	1.027	1.090	1.058	17330	0.221784	0.008603	0.0388
Liua tsinpaensis	0	16380	ATP6	7960	8643	0.339	0.423	0.381	6241	0.200076	0.026822	0.0001
Liua tsinpaensis	0	16380	ATP8	7802	7969	0.320	0.340	0.330	5409	0.142141	0.000014	0.0001
Liua tsinpaensis	0	16380	COX1	5349	6899	0.021	0.210	0.115	1886	0.266096	0.006097	0.0229
Liua tsinpaensis	0	16380	COX2	7042	7729	0.227	0.311	0.269	4409	0.191674	0.004024	0.021
Liua tsinpaensis	0	16380	COX3	8643	9426	0.423	0.518	0.471	7707	0.216655	0.008678	0.0401
Liua tsinpaensis	0	16380	CYTB	14181	15321	1.099	1.238	1.169	19140	0.307365	0.017494	0.0569
Liua tsinpaensis	0	16380	ND1	2747	3714	0.703	0.821	0.762	12479	0.42486	0.015089	0.0355
Liua tsinpaensis	0	16380	ND2	3925	4966	0.847	0.974	0.910	14908	0.231084	0.012727	0.0551
Liua tsinpaensis	0	16380	ND3	9496	9846	0.527	0.570	0.548	8980	0.109819	0.005012	0.0456
Liua tsinpaensis	0	16380	ND4	10204	11578	0.613	0.781	0.697	11420	0.297256	0.015049	0.0506
Liua tsinpaensis	0	16380	ND4L	9914	10210	0.578	0.614	0.596	9762	0.16403	0.01489	0.0908
Liua tsinpaensis	0	16380	ND5	11787	13604	0.807	1.028	0.918	15029	0.287688	0.013654	0.0475
Liua tsinpaensis	0	16380	ND6	13590	14108	1.027	1.090	1.058	17336	0.359256	0.018365	0.0511

<i>Lyciasalamandra atifi</i>	0	16650	ATP6	7912	8595	0.329	0.411	0.370	6153	1.72165	0.082276	0.0598
<i>Lyciasalamandra atifi</i>	0	16650	ATP8	7754	7921	0.310	0.330	0.320	5321	0.207211	0.012386	0.0598
<i>Lyciasalamandra atifi</i>	0	16650	COX1	5301	6860	0.015	0.202	0.109	1807	1.126182	0.005618	0.005
<i>Lyciasalamandra atifi</i>	0	16650	COX2	6993	7680	0.218	0.301	0.259	4319	0.908607	0.008272	0.0091
<i>Lyciasalamandra atifi</i>	0	16650	COX3	8595	9378	0.411	0.505	0.458	7619	0.695724	0.014951	0.0215
<i>Lyciasalamandra atifi</i>	0	16650	CYTB	14126	15266	1.075	1.212	1.143	19038	0.91937	0.044923	0.0489
<i>Lyciasalamandra atifi</i>	0	16650	ND1	2702	3673	0.703	0.819	0.761	12671	1.378744	0.028509	0.0207
<i>Lyciasalamandra atifi</i>	0	16650	ND2	3886	4923	0.845	0.970	0.907	15105	1.304378	0.068643	0.0526
<i>Lyciasalamandra atifi</i>	0	16650	ND3	9447	9792	0.513	0.554	0.534	8885	0.594465	0.000059	0.0001
<i>Lyciasalamandra atifi</i>	0	16650	ND4	10152	11529	0.598	0.763	0.680	11327	1.101563	0.048188	0.0437
<i>Lyciasalamandra atifi</i>	0	16650	ND4L	9862	10158	0.563	0.598	0.581	9666	1.37116	0.063264	0.0461
<i>Lyciasalamandra atifi</i>	0	16650	ND5	11738	13552	0.788	1.006	0.897	14936	1.527918	0.068722	0.045
<i>Lyciasalamandra atifi</i>	0	16650	ND6	13538	14053	1.004	1.066	1.035	17237	1.526218	0.072641	0.0476
<i>Oedipina poelzi</i>	0	16731	ATP6	7797	8480	0.330	0.412	0.371	6201	1.756016	0.072597	0.136
<i>Oedipina poelzi</i>	0	16731	ATP8	7645	7806	0.312	0.331	0.321	5375	0.545204	0.074138	0.136
<i>Oedipina poelzi</i>	0	16731	COX1	5206	6753	0.020	0.205	0.113	1883	1.557983	0.018076	0.0116
<i>Oedipina poelzi</i>	0	16731	COX2	6886	7573	0.221	0.303	0.262	4383	1.295458	0.034601	0.0267
<i>Oedipina poelzi</i>	0	16731	COX3	8480	9263	0.412	0.505	0.458	7668	1.695378	0.027524	0.0162
<i>Oedipina poelzi</i>	0	16731	CYTB	14007	15147	1.072	1.208	1.140	19078	1.269902	0.052487	0.0413
<i>Oedipina poelzi</i>	0	16731	ND1	2614	3585	0.710	0.826	0.768	12854	1.26085	0.029144	0.0231
<i>Oedipina poelzi</i>	0	16731	ND2	3791	4828	0.851	0.975	0.913	15274	1.75718	0.115329	0.0656
<i>Oedipina poelzi</i>	0	16731	ND3	9329	9676	0.513	0.554	0.534	8928	2.309045	0.07705	0.0334
<i>Oedipina poelzi</i>	0	16731	ND4	10033	11407	0.597	0.761	0.679	11364	1.345849	0.060705	0.0451

Oedipina poelzi	0	16731	ND4L	9743	10039	0.562	0.598	0.580	9706	1.013503	0.04353	0.0429
Oedipina poelzi	0	16731	ND5	11613	13416	0.786	1.002	0.894	14953	1.355179	0.050924	0.0376
Oedipina poelzi	0	16731	ND6	13419	13937	1.002	1.064	1.033	17281	0.853472	0.057996	0.068
Onychodactylus fischeri	0	16456	ATP6	7942	8625	0.338	0.421	0.380	6248	1.702476	0.075796	0.161
Onychodactylus fischeri	0	16456	ATP8	7784	7951	0.319	0.339	0.329	5416	0.549643	0.088494	0.161
Onychodactylus fischeri	0	16456	COX1	5328	6878	0.021	0.209	0.115	1888	1.432003	0.009643	0.0067
Onychodactylus fischeri	0	16456	COX2	7024	7710	0.227	0.310	0.268	4416	0.807661	0.013947	0.0173
Onychodactylus fischeri	0	16456	COX3	8625	9409	0.421	0.517	0.469	7715	1.298388	0.033103	0.0255
Onychodactylus fischeri	0	16456	CYTB	14162	15299	1.094	1.232	1.163	19143	1.290264	0.033118	0.0257
Onychodactylus fischeri	0	16456	ND1	2721	3692	0.704	0.822	0.763	12551	1.517754	0.044093	0.0291
Onychodactylus fischeri	0	16456	ND2	3902	4942	0.847	0.974	0.910	14982	1.031079	0.052608	0.051
Onychodactylus fischeri	0	16456	ND3	9477	9827	0.525	0.567	0.546	8986	1.734999	0.056535	0.0326
Onychodactylus fischeri	0	16456	ND4	10185	11562	0.611	0.778	0.695	11429	1.070603	0.040253	0.0376
Onychodactylus fischeri	0	16456	ND4L	9895	10191	0.576	0.612	0.594	9768	1.075115	0.05628	0.0523
Onychodactylus fischeri	0	16456	ND5	11772	13586	0.804	1.024	0.914	15040	1.499934	0.064094	0.0427
Onychodactylus fischeri	0	16456	ND6	13569	14090	1.022	1.085	1.054	17341	0.844671	0.032338	0.0383
Pachyhynobius shangchengensis	0	16394	ATP6	7941	8624	0.339	0.422	0.380	6237	0.593031	0.03031	0.1106
Pachyhynobius shangchengensis	0	16394	ATP8	7783	7950	0.320	0.340	0.330	5405	0.355827	0.039366	0.1106
Pachyhynobius shangchengensis	0	16394	COX1	5330	6880	0.020	0.209	0.115	1882	0.742862	0.00433	0.0058
Pachyhynobius shangchengensis	0	16394	COX2	7024	7710	0.227	0.311	0.269	4406	0.399465	0.009734	0.0244

Pachyhynobius shangchengensis	0	16394	COX3	8624	9409	0.422	0.518	0.470	7705	0.497862	0.016813	0.0338
Pachyhynobius shangchengensis	0	16394	CYTB	14169	15310	1.099	1.238	1.168	19151	0.717167	0.026634	0.0371
Pachyhynobius shangchengensis	0	16394	ND1	2728	3699	0.703	0.821	0.762	12493	0.610784	0.019384	0.0317
Pachyhynobius shangchengensis	0	16394	ND2	3909	4952	0.847	0.974	0.911	14927	0.582351	0.044928	0.0771
Pachyhynobius shangchengensis	0	16394	ND3	9478	9828	0.526	0.569	0.548	8978	0.775185	0.013972	0.018
Pachyhynobius shangchengensis	0	16394	ND4	10185	11562	0.613	0.781	0.697	11418	0.856823	0.043036	0.0502
Pachyhynobius shangchengensis	0	16394	ND4L	9895	10191	0.577	0.613	0.595	9759	0.801446	0.048656	0.0607
Pachyhynobius shangchengensis	0	16394	ND5	11772	13592	0.806	1.028	0.917	15036	0.875103	0.038575	0.0441
Pachyhynobius shangchengensis	0	16394	ND6	13578	14096	1.027	1.090	1.058	17346	0.486207	0.01311	0.027
Paradactylodon gorganensis	0	16374	ATP6	7971	8654	0.339	0.423	0.381	6241	0.490158	0.030437	0.2969
Paradactylodon gorganensis	0	16374	ATP8	7813	7980	0.320	0.341	0.330	5408	0.071154	0.021127	0.2969
Paradactylodon gorganensis	0	16374	COX1	5361	6911	0.021	0.210	0.115	1888	0.391531	0.005377	0.0137
Paradactylodon gorganensis	0	16374	COX2	7054	7741	0.227	0.311	0.269	4410	0.368391	0.008021	0.0218
Paradactylodon gorganensis	0	16374	COX3	8654	9438	0.423	0.519	0.471	7708	0.561396	0.013545	0.0241
Paradactylodon gorganensis	0	16374	CYTB	14198	15339	1.100	1.239	1.170	19153	0.682839	0.023174	0.0339
Paradactylodon gorganensis	0	16374	ND1	2756	3727	0.703	0.821	0.762	12474	0.51783	0.029783	0.0575
Paradactylodon gorganensis	0	16374	ND2	3936	4979	0.847	0.974	0.910	14905	0.417548	0.027408	0.0656
Paradactylodon gorganensis	0	16374	ND3	9506	9856	0.527	0.570	0.548	8978	0.573933	0.031025	0.0541
Paradactylodon gorganensis	0	16374	ND4	10214	11591	0.613	0.782	0.698	11421	0.387703	0.019035	0.0491

Paradactylodon gorganensis	0	16374	ND4L	9924	10220	0.578	0.614	0.596	9760	0.443485	0.004178	0.0094
Paradactylodon gorganensis	0	16374	ND5	11801	13622	0.807	1.030	0.919	15040	0.531949	0.040103	0.0754
Paradactylodon gorganensis	0	16374	ND6	13607	14125	1.028	1.091	1.059	17347	0.380883	0.036468	0.0957
Paradactylodon mustersi	0	16383	ATP6	7966	8649	0.339	0.423	0.381	6239	0.341533	0.00867	0.0653
Paradactylodon mustersi	0	16383	ATP8	7808	7975	0.320	0.340	0.330	5406	0.294354	0.019221	0.0653
Paradactylodon mustersi	0	16383	COX1	5356	6906	0.021	0.210	0.115	1886	0.490105	0.004424	0.009
Paradactylodon mustersi	0	16383	COX2	7049	7736	0.227	0.311	0.269	4409	0.467334	0.003791	0.0081
Paradactylodon mustersi	0	16383	COX3	8649	9433	0.423	0.518	0.470	7706	0.180249	0.00172	0.0095
Paradactylodon mustersi	0	16383	CYTB	14189	15330	1.099	1.238	1.168	19143	0.575282	0.011076	0.0193
Paradactylodon mustersi	0	16383	ND1	2751	3722	0.703	0.821	0.762	12480	0.56154	0.011812	0.021
Paradactylodon mustersi	0	16383	ND2	3932	4975	0.847	0.974	0.910	14914	0.486054	0.028947	0.0596
Paradactylodon mustersi	0	16383	ND3	9502	9852	0.527	0.569	0.548	8978	0.759333	0.024456	0.0322
Paradactylodon mustersi	0	16383	ND4	10210	11587	0.613	0.781	0.697	11421	0.553793	0.039925	0.0721
Paradactylodon mustersi	0	16383	ND4L	9920	10216	0.578	0.614	0.596	9760	0.398574	0.030372	0.0762
Paradactylodon mustersi	0	16383	ND5	11796	13614	0.807	1.029	0.918	15034	0.509181	0.014654	0.0288
Paradactylodon mustersi	0	16383	ND6	13599	14117	1.027	1.090	1.058	17340	0.281198	0.014059	0.05
Paramesotriton hongkongensis	0	16285	ATP6	7906	8589	0.341	0.425	0.383	6243	0.746067	0.051623	0.1531
Paramesotriton hongkongensis	0	16285	ATP8	7748	7915	0.322	0.343	0.332	5411	0.601578	0.092114	0.1531
Paramesotriton hongkongensis	0	16285	COX1	5294	6853	0.021	0.212	0.116	1895	0.801889	0.004784	0.006

Paramesotriton hongkongensis	0	16285	COX2	6986	7673	0.228	0.313	0.271	4407	0.466948	0.00861	0.0184
Paramesotriton hongkongensis	0	16285	COX3	8589	9372	0.425	0.522	0.473	7709	0.677351	0.018356	0.0271
Paramesotriton hongkongensis	0	16285	CYTB	14113	15253	1.104	1.244	1.174	19114	0.88556	0.049345	0.0557
Paramesotriton hongkongensis	0	16285	ND1	2702	3670	0.702	0.821	0.762	12405	0.584276	0.029311	0.0502
Paramesotriton hongkongensis	0	16285	ND2	3882	4916	0.847	0.974	0.911	14831	0.919246	0.07422	0.0807
Paramesotriton hongkongensis	0	16285	ND3	9442	9789	0.530	0.573	0.551	8980	1.519376	0.045248	0.0298
Paramesotriton hongkongensis	0	16285	ND4	10146	11526	0.617	0.786	0.701	11420	0.878123	0.039118	0.0445
Paramesotriton hongkongensis	0	16285	ND4L	9856	10152	0.581	0.617	0.599	9756	0.775652	0.048738	0.0628
Paramesotriton hongkongensis	0	16285	ND5	11728	13539	0.811	1.033	0.922	15015	0.74919	0.042454	0.0567
Paramesotriton hongkongensis	0	16285	ND6	13525	14043	1.032	1.095	1.063	17316	0.818022	0.021431	0.0262
Phaeognathus hubrichti	0	16294	ATP6	7896	8579	0.341	0.425	0.383	6235	0.445537	0.037404	0.0681
Phaeognathus hubrichti	0	16294	ATP8	7741	7905	0.322	0.342	0.332	5406	0.634401	0.043195	0.0681
Phaeognathus hubrichti	0	16294	COX1	5289	6832	0.021	0.210	0.115	1880	0.621345	0.003765	0.0061
Phaeognathus hubrichti	0	16294	COX2	6980	7667	0.228	0.313	0.270	4407	0.420328	0.01874	0.0446
Phaeognathus hubrichti	0	16294	COX3	8579	9362	0.425	0.521	0.473	7701	0.691693	0.014799	0.0214
Phaeognathus hubrichti	0	16294	CYTB	14101	15241	1.102	1.242	1.172	19102	0.686266	0.021924	0.0319
Phaeognathus hubrichti	0	16294	ND1	2688	3659	0.702	0.821	0.761	12401	0.532946	0.019808	0.0372
Phaeognathus hubrichti	0	16294	ND2	3867	4904	0.846	0.974	0.910	14825	0.680992	0.044919	0.066
Phaeognathus hubrichti	0	16294	ND3	9430	9777	0.529	0.572	0.550	8967	0.380455	0.025625	0.0674

Phaeognathus hubrichti	0	16294	ND4	10134	11505	0.615	0.784	0.700	11398	0.76055	0.029119	0.0383
Phaeognathus hubrichti	0	16294	ND4L	9844	10140	0.580	0.616	0.598	9744	0.576739	0.03532	0.0612
Phaeognathus hubrichti	0	16294	ND5	11713	13524	0.809	1.032	0.920	14997	0.590077	0.028369	0.0481
Phaeognathus hubrichti	0	16294	ND6	13510	14028	1.030	1.093	1.062	17298	0.63008	0.031306	0.0497
Plethodon cinereus	1	20001	ATP6	8044	8727	0.300	0.368	0.334	6685	1.365125	0.075654	0.131
Plethodon cinereus	1	20001	ATP8	7889	8053	0.285	0.301	0.293	5856	0.357224	0.046799	0.131
Plethodon cinereus	1	20001	COX1	5212	6759	0.017	0.172	0.094	1885	0.5775	0.012371	0.0214
Plethodon cinereus	1	20001	COX2	6894	7700	0.185	0.266	0.225	4508	0.754472	0.051914	0.0688
Plethodon cinereus	1	20001	COX3	8727	9510	0.368	0.447	0.408	8151	0.396096	0.030832	0.0778
Plethodon cinereus	1	20001	CYTB	14229	15366	0.919	1.032	0.975	19509	0.631163	0.034876	0.0553
Plethodon cinereus	1	20001	ND1	2613	3581	0.757	0.854	0.805	16109	0.653896	0.033522	0.0513
Plethodon cinereus	1	20001	ND2	3792	4832	0.875	0.979	0.927	18539	0.797778	0.058756	0.0736
Plethodon cinereus	1	20001	ND3	9577	9924	0.453	0.488	0.471	9415	0.572749	0.022539	0.0394
Plethodon cinereus	1	20001	ND4	10281	11630	0.524	0.659	0.591	11826	0.664559	0.039105	0.0588
Plethodon cinereus	1	20001	ND4L	9991	10287	0.495	0.524	0.510	10193	0.691301	0.075983	0.1099
Plethodon cinereus	1	20001	ND5	11843	13663	0.680	0.862	0.771	15421	0.696379	0.038057	0.0546
Plethodon cinereus	1	20001	ND6	13646	14158	0.860	0.912	0.886	17719	0.572047	0.03295	0.0576
Plethodon elongatus	1	18767	ATP6	7844	8527	0.295	0.368	0.331	6218	0.767369	0.045231	0.2086
Plethodon elongatus	1	18767	ATP8	7686	7853	0.278	0.296	0.287	5384	0.470862	0.098235	0.2086
Plethodon elongatus	1	18767	COX1	5245	6784	0.018	0.182	0.100	1875	0.55128	0.00589	0.0107
Plethodon elongatus	1	18767	COX2	6925	7606	0.197	0.270	0.233	4376	0.700763	0.035203	0.0502
Plethodon elongatus	1	18767	COX3	8527	9310	0.368	0.451	0.409	7683	0.531054	0.019537	0.0368
Plethodon elongatus	1	18767	CYTB	15341	16483	1.094	1.216	1.155	21669	0.750927	0.035563	0.0474
Plethodon elongatus	1	18767	ND1	2649	3617	0.741	0.844	0.793	14878	0.784835	0.031744	0.0404
Plethodon elongatus	1	18767	ND2	3826	4866	0.867	0.978	0.922	17305	0.661201	0.052187	0.0789
Plethodon elongatus	1	18767	ND3	9378	9725	0.458	0.495	0.477	8949	0.587916	0.019928	0.0339
Plethodon elongatus	1	18767	ND4	10081	11455	0.533	0.680	0.607	11382	0.699843	0.042372	0.0605
Plethodon elongatus	1	18767	ND4L	9791	10087	0.502	0.534	0.518	9724	0.724461	0.063917	0.0882

Plethodon elongatus	1	18767	ND5	11665	13477	0.702	0.895	0.799	14988	0.71451	0.044137	0.0618
Plethodon elongatus	1	18767	ND6	14762	15271	1.032	1.086	1.059	19879	0.722622	0.053391	0.0739
Plethodon petraeus	1	19235	ATP6	7772	8455	0.285	0.356	0.321	6165	1.07532	0.07569	0.2421
Plethodon petraeus	1	19235	ATP8	7617	7781	0.269	0.286	0.277	5336	0.961673	0.232869	0.2421
Plethodon petraeus	1	19235	COX1	5195	6745	0.017	0.178	0.098	1878	0.505807	0.012629	0.025
Plethodon petraeus	1	19235	COX2	6880	7539	0.192	0.261	0.227	4358	0.658711	0.035413	0.0538
Plethodon petraeus	1	19235	COX3	8455	9238	0.356	0.437	0.397	7631	0.528673	0.025833	0.0489
Plethodon petraeus	1	19235	CYTB	13952	15089	0.928	1.046	0.987	18979	0.850757	0.045869	0.0539
Plethodon petraeus	1	19235	ND1	2607	3573	0.748	0.848	0.798	15353	0.844273	0.034679	0.0411
Plethodon petraeus	1	19235	ND2	3780	4820	0.870	0.978	0.924	17773	0.805208	0.062138	0.0772
Plethodon petraeus	1	19235	ND3	9305	9652	0.444	0.481	0.462	8895	0.919919	0.063415	0.0689
Plethodon petraeus	1	19235	ND4	10009	11358	0.518	0.658	0.588	11305	0.63742	0.032278	0.0506
Plethodon petraeus	1	19235	ND4L	9719	10015	0.487	0.518	0.503	9671	0.464442	0.037005	0.0797
Plethodon petraeus	1	19235	ND5	11570	13381	0.680	0.868	0.774	14889	0.668525	0.034744	0.052
Plethodon petraeus	1	19235	ND6	13367	13882	0.867	0.920	0.894	17187	1.068895	0.061364	0.0574
Pseudotriton ruber	0	16661	ATP6	7857	8540	0.333	0.415	0.374	6223	0.570201	0.041674	0.1989
Pseudotriton ruber	0	16661	ATP8	7702	7866	0.314	0.334	0.324	5394	0.147838	0.02941	0.1989
Pseudotriton ruber	0	16661	COX1	5254	6807	0.020	0.207	0.113	1887	0.568208	0.00356	0.0063
Pseudotriton ruber	0	16661	COX2	6942	7623	0.223	0.304	0.264	4391	0.341362	0.006395	0.0187
Pseudotriton ruber	0	16661	COX3	8540	9323	0.415	0.509	0.462	7689	0.43628	0.013465	0.0309
Pseudotriton ruber	0	16661	CYTB	14057	15198	1.077	1.214	1.145	19081	0.430516	0.013083	0.0304
Pseudotriton ruber	0	16661	ND1	2667	3623	0.710	0.824	0.767	12777	0.734321	0.017225	0.0235
Pseudotriton ruber	0	16661	ND2	3838	4878	0.850	0.975	0.913	15203	0.584668	0.036439	0.0623
Pseudotriton ruber	0	16661	ND3	9392	9739	0.517	0.558	0.538	8957	0.687857	0.035795	0.052
Pseudotriton ruber	0	16661	ND4	10097	11470	0.601	0.766	0.684	11393	0.665131	0.025962	0.039
Pseudotriton ruber	0	16661	ND4L	9807	10103	0.567	0.602	0.584	9736	0.688968	0.025824	0.0375
Pseudotriton ruber	0	16661	ND5	11678	13486	0.791	1.008	0.900	14990	0.491493	0.01763	0.0359
Pseudotriton ruber	0	16661	ND6	13472	13990	1.007	1.069	1.038	17287	0.699409	0.044871	0.0642
Ranodon sibiricus	0	16418	ATP6	7966	8649	0.339	0.422	0.380	6240	0.285944	0.010471	0.1494
Ranodon sibiricus	0	16418	ATP8	7808	7975	0.319	0.340	0.329	5409	0.187734	0.028039	0.1494

Ranodon sibiricus	0	16418	COX1	5355	6905	0.021	0.209	0.115	1886	0.416781	0.00084	0.002
Ranodon sibiricus	0	16418	COX2	7049	7736	0.227	0.311	0.269	4411	0.286045	0.003868	0.0135
Ranodon sibiricus	0	16418	COX3	8649	9432	0.422	0.517	0.469	7707	0.497716	0.015965	0.0321
Ranodon sibiricus	0	16418	CYTB	14182	15322	1.096	1.235	1.165	19129	0.423726	0.010942	0.0258
Ranodon sibiricus	0	16418	ND1	2752	3721	0.703	0.821	0.762	12517	0.444112	0.015479	0.0349
Ranodon sibiricus	0	16418	ND2	3932	4975	0.847	0.974	0.911	14951	0.326039	0.012257	0.0376
Ranodon sibiricus	0	16418	ND3	9502	9852	0.526	0.568	0.547	8980	0.43891	0.01943	0.0443
Ranodon sibiricus	0	16418	ND4	10210	11581	0.612	0.779	0.695	11417	0.518021	0.019116	0.0369
Ranodon sibiricus	0	16418	ND4L	9920	10216	0.577	0.613	0.595	9762	0.640804	0.013079	0.0204
Ranodon sibiricus	0	16418	ND5	11794	13617	0.805	1.027	0.916	15036	0.500762	0.0261	0.0521
Ranodon sibiricus	0	16418	ND6	13591	14109	1.024	1.087	1.055	17327	0.147086	0.005366	0.0365
Rhyacotriton variegatus	1	21606	ATP6	7864	8547	0.257	0.321	0.289	6241	2.872768	0.143002	0.266
Rhyacotriton variegatus	1	21606	ATP8	7709	7873	0.243	0.258	0.251	5412	0.65793	0.175018	0.266
Rhyacotriton variegatus	1	21606	COX1	5254	6802	0.016	0.159	0.087	1885	2.744934	0.025231	0.0092
Rhyacotriton variegatus	1	21606	COX2	6946	7629	0.172	0.236	0.204	4405	2.070658	0.046645	0.0225
Rhyacotriton variegatus	1	21606	COX3	8547	9330	0.321	0.393	0.357	7707	1.878426	0.059186	0.0315
Rhyacotriton variegatus	1	21606	CYTB	14044	15184	0.829	0.935	0.882	19058	1.888065	0.072596	0.0384
Rhyacotriton variegatus	1	21606	ND1	2650	3619	0.775	0.864	0.819	17705	2.280558	0.06517	0.0286
Rhyacotriton variegatus	1	21606	ND2	3829	4872	0.884	0.980	0.932	20137	2.269425	0.124774	0.055
Rhyacotriton variegatus	1	21606	ND3	9394	9741	0.399	0.431	0.415	8965	2.314175	0.066497	0.0287
Rhyacotriton variegatus	1	21606	ND4	10098	11460	0.464	0.590	0.527	11387	1.978635	0.079265	0.0401
Rhyacotriton variegatus	1	21606	ND4L	9808	10104	0.437	0.465	0.451	9742	1.960786	0.078819	0.0402
Rhyacotriton variegatus	1	21606	ND5	11664	13478	0.609	0.777	0.693	14972	2.029494	0.082984	0.0409

Rhyacotriton variegatus	1	21606	ND6	13454	13972	0.775	0.823	0.799	17256	1.913713	0.071437	0.0373
Salamandrella keyserlingii	0	16338	ATP6	7969	8652	0.340	0.424	0.382	6244	0.451216	0.01307	0.2235
Salamandrella keyserlingii	0	16338	ATP8	7811	7978	0.321	0.342	0.331	5413	0.350264	0.078287	0.2235
Salamandrella keyserlingii	0	16338	COX1	5359	6909	0.021	0.211	0.116	1892	0.823551	0.005264	0.0064
Salamandrella keyserlingii	0	16338	COX2	7053	7740	0.228	0.312	0.270	4417	0.614508	0.005827	0.0095
Salamandrella keyserlingii	0	16338	COX3	8652	9436	0.424	0.520	0.472	7712	0.485912	0.001856	0.0038
Salamandrella keyserlingii	0	16338	CYTB	14196	15337	1.103	1.242	1.173	19157	0.824768	0.034023	0.0413
Salamandrella keyserlingii	0	16338	ND1	2751	3722	0.702	0.821	0.761	12435	0.714134	0.008086	0.0113
Salamandrella keyserlingii	0	16338	ND2	3932	4975	0.846	0.974	0.910	14868	0.874676	0.032904	0.0376
Salamandrella keyserlingii	0	16338	ND3	9504	9852	0.528	0.571	0.550	8979	0.477845	0.02584	0.0541
Salamandrella keyserlingii	0	16338	ND4	10212	11589	0.615	0.784	0.699	11425	0.812105	0.034239	0.0422
Salamandrella keyserlingii	0	16338	ND4L	9922	10218	0.580	0.616	0.598	9764	0.49248	0.023681	0.0481
Salamandrella keyserlingii	0	16338	ND5	11799	13619	0.809	1.032	0.921	15042	0.758533	0.03787	0.0499
Salamandrella keyserlingii	0	16338	ND6	13605	14123	1.030	1.094	1.062	17353	0.720095	0.033013	0.0458
Stereochilus marginatus	1	19631	ATP6	7872	8555	0.283	0.352	0.317	6231	0.608072	0.036117	0.11
Stereochilus marginatus	1	19631	ATP8	7717	7881	0.267	0.284	0.275	5401	0.56959	0.062656	0.11
Stereochilus marginatus	1	19631	COX1	5267	6820	0.017	0.175	0.096	1890	0.384791	0.000922	0.0024
Stereochilus marginatus	1	19631	COX2	6955	7639	0.189	0.259	0.224	4398	0.439939	0.00525	0.0119
Stereochilus marginatus	1	19631	COX3	8555	9338	0.352	0.432	0.392	7697	0.516866	0.010088	0.0195

Stereochilus marginatus	1	19631	CYTB	13761	14901	0.883	0.999	0.941	18466	0.603127	0.020145	0.0334
Stereochilus marginatus	1	19631	ND1	2677	3636	0.753	0.851	0.802	15748	0.643092	0.023173	0.036
Stereochilus marginatus	1	19631	ND2	3849	4889	0.873	0.979	0.926	18173	0.623853	0.025599	0.041
Stereochilus marginatus	1	19631	ND3	9406	9753	0.439	0.474	0.457	8963	0.812374	0.030059	0.037
Stereochilus marginatus	1	19631	ND4	10110	11484	0.511	0.651	0.581	11398	0.565221	0.027999	0.0495
Stereochilus marginatus	1	19631	ND4L	9820	10116	0.481	0.511	0.496	9740	0.440641	0.021184	0.0481
Stereochilus marginatus	1	19631	ND5	11691	13505	0.672	0.857	0.764	15000	0.524064	0.031826	0.0607
Stereochilus marginatus	1	19631	ND6	16951	17466	1.208	1.260	1.234	24221	0.379225	0.017165	0.0453
Thorius sp	1	19097	ATP6	7806	8489	0.289	0.360	0.325	6199	1.483668	0.078908	0.1561
Thorius sp	1	19097	ATP8	7654	7815	0.273	0.290	0.281	5373	0.401249	0.062635	0.1561
Thorius sp	1	19097	COX1	5215	6756	0.018	0.179	0.098	1875	0.846961	0.009433	0.0111
Thorius sp	1	19097	COX2	6896	7583	0.194	0.266	0.230	4383	0.560643	0.017081	0.0305
Thorius sp	1	19097	COX3	8489	9272	0.360	0.442	0.401	7666	0.751208	0.018391	0.0245
Thorius sp	1	19097	CYTB	14015	15155	0.939	1.059	0.999	19074	1.288701	0.042943	0.0333
Thorius sp	1	19097	ND1	2630	3595	0.747	0.848	0.797	15226	1.147887	0.034375	0.0299
Thorius sp	1	19097	ND2	3805	4842	0.870	0.978	0.924	17648	0.818118	0.069104	0.0845
Thorius sp	1	19097	ND3	9339	9686	0.449	0.486	0.468	8929	1.641248	0.07933	0.0483
Thorius sp	1	19097	ND4	10042	11413	0.523	0.667	0.595	11359	1.185314	0.046522	0.0392
Thorius sp	1	19097	ND4L	9752	10048	0.493	0.524	0.508	9703	0.950416	0.035392	0.0372
Thorius sp	1	19097	ND5	11624	13447	0.689	0.880	0.784	14975	0.991598	0.055378	0.0558
Thorius sp	1	19097	ND6	13430	13948	0.878	0.932	0.905	17282	1.272957	0.035701	0.028
Triturus carnifex	0	16560	ATP6	7909	8592	0.332	0.415	0.373	6181	0.074674	0.008439	--
Triturus carnifex	0	16560	ATP8	7751	7918	0.313	0.333	0.323	5349	0.000005	0	--
Triturus carnifex	0	16560	COX1	5296	6846	0.016	0.204	0.110	1822	0.035184	0.00307	0.0873
Triturus carnifex	0	16560	COX2	6989	7676	0.221	0.304	0.262	4345	0.081127	0.000008	0.0001
Triturus carnifex	0	16560	COX3	8592	9375	0.415	0.509	0.462	7647	0.036417	0.007531	0.2068

Triturus carnifex	0	16560	CYTB	14126	15266	1.083	1.221	1.152	19072	0.066888	0.010977	0.1641
Triturus carnifex	0	16560	ND1	2699	3665	0.703	0.819	0.761	12604	0.084036	0.004645	0.0553
Triturus carnifex	0	16560	ND2	3878	4909	0.845	0.970	0.907	15027	0.117199	0.00634	0.0541
Triturus carnifex	0	16560	ND3	9445	9792	0.518	0.559	0.538	8917	0.094418	0.005708	0.0605
Triturus carnifex	0	16560	ND4	10150	11527	0.603	0.769	0.686	11358	0.071559	0.01178	0.1646
Triturus carnifex	0	16560	ND4L	9860	10156	0.568	0.603	0.586	9696	0.017404	0.000002	0.0001
Triturus carnifex	0	16560	ND5	11738	13552	0.794	1.014	0.904	14969	0.052381	0.0051	0.0974
Triturus carnifex	0	16560	ND6	13538	14056	1.012	1.074	1.043	17274	0.064923	0.000006	0.0001
Triturus cristatus	0	16564	ATP6	7910	8593	0.332	0.414	0.373	6181	0.158006	0.00703	0.0001
Triturus cristatus	0	16564	ATP8	7752	7919	0.313	0.333	0.323	5349	0.050898	0.000005	0.0001
Triturus cristatus	0	16564	COX1	5297	6847	0.016	0.204	0.110	1822	0.150493	0.000983	0.0065
Triturus cristatus	0	16564	COX2	6990	7677	0.221	0.304	0.262	4345	0.110995	0.000011	0.0001
Triturus cristatus	0	16564	COX3	8593	9376	0.414	0.509	0.462	7647	0.104207	0.005497	0.0527
Triturus cristatus	0	16564	CYTB	14125	15265	1.082	1.220	1.151	19068	0.159027	0.010684	0.0672
Triturus cristatus	0	16564	ND1	2700	3666	0.703	0.820	0.761	12608	0.151845	0.00313	0.0206
Triturus cristatus	0	16564	ND2	3879	4913	0.845	0.970	0.908	15034	0.195557	0.008146	0.0417
Triturus cristatus	0	16564	ND3	9446	9793	0.517	0.559	0.538	8917	0.136201	0.000014	0.0001
Triturus cristatus	0	16564	ND4	10151	11528	0.603	0.769	0.686	11357	0.182612	0.009036	0.0495
Triturus cristatus	0	16564	ND4L	9861	10157	0.568	0.603	0.585	9696	0.113987	0.014959	0.1312
Triturus cristatus	0	16564	ND5	11737	13551	0.794	1.013	0.904	14966	0.121737	0.007685	0.0631
Triturus cristatus	0	16564	ND6	13537	14055	1.011	1.074	1.043	17270	0.176693	0.009543	0.054
Triturus dobrogicus	0	16425	ATP6	7910	8593	0.335	0.418	0.376	6181	0.110325	0.007116	0.0001
Triturus dobrogicus	0	16425	ATP8	7752	7919	0.316	0.336	0.326	5349	0.155408	0.000016	0.0001
Triturus dobrogicus	0	16425	COX1	5297	6847	0.017	0.205	0.111	1822	0.141806	0.001993	0.0141
Triturus dobrogicus	0	16425	COX2	6990	7677	0.223	0.306	0.265	4345	0.19795	0.00002	0.0001
Triturus dobrogicus	0	16425	COX3	8593	9376	0.418	0.513	0.466	7647	0.083009	0.001798	0.0217
Triturus dobrogicus	0	16425	CYTB	14126	15266	1.092	1.230	1.161	19069	0.199924	0.003827	0.0191
Triturus dobrogicus	0	16425	ND1	2699	3665	0.700	0.818	0.759	12467	0.14313	0.006338	0.0443
Triturus dobrogicus	0	16425	ND2	3878	4912	0.844	0.970	0.907	14893	0.143376	0.006463	0.0451
Triturus dobrogicus	0	16425	ND3	9446	9793	0.522	0.564	0.543	8917	0.097751	0.011718	0.1199

Triturus dobrogicus	0	16425	ND4	10151	11528	0.608	0.775	0.691	11357	0.10222	0.004594	0.0449
Triturus dobrogicus	0	16425	ND4L	9861	10157	0.572	0.608	0.590	9696	0.152552	0.014623	0.0959
Triturus dobrogicus	0	16425	ND5	11738	13552	0.801	1.022	0.911	14968	0.140537	0.011834	0.0842
Triturus dobrogicus	0	16425	ND6	13538	14056	1.020	1.083	1.052	17272	0.264188	0.018767	0.071
Triturus karelinii	0	16541	ATP6	7911	8594	0.332	0.415	0.374	6181	0.081594	0.002441	0.0001
Triturus karelinii	0	16541	ATP8	7753	7920	0.313	0.334	0.323	5349	0.106962	0.000011	0.0001
Triturus karelinii	0	16541	COX1	5298	6848	0.016	0.204	0.110	1822	0.118253	0.000012	0.0001
Triturus karelinii	0	16541	COX2	6991	7678	0.221	0.304	0.263	4344	0.104914	0.00225	0.0214
Triturus karelinii	0	16541	COX3	8594	9377	0.415	0.510	0.462	7647	0.06808	0.003748	0.0551
Triturus karelinii	0	16541	CYTB	14127	15267	1.084	1.222	1.153	19070	0.098615	0.005416	0.0549
Triturus karelinii	0	16541	ND1	2699	3665	0.702	0.819	0.761	12581	0.188859	0.007993	0.0423
Triturus karelinii	0	16541	ND2	3878	4912	0.845	0.970	0.907	15007	0.190486	0.013204	0.0693
Triturus karelinii	0	16541	ND3	9447	9794	0.518	0.560	0.539	8917	0.137383	0.005575	0.0406
Triturus karelinii	0	16541	ND4	10152	11529	0.603	0.770	0.687	11356	0.103689	0.014028	0.1353
Triturus karelinii	0	16541	ND4L	9862	10158	0.568	0.604	0.586	9696	0.142787	0.015379	0.1077
Triturus karelinii	0	16541	ND5	11739	13553	0.795	1.015	0.905	14968	0.122894	0.011096	0.0903
Triturus karelinii	0	16541	ND6	13539	14057	1.013	1.076	1.044	17272	0.180771	0.014583	0.0807
Triturus macedonicus	0	16569	ATP6	7911	8594	0.332	0.414	0.373	6181	0.086973	0.008713	--
Triturus macedonicus	0	16569	ATP8	7753	7920	0.313	0.333	0.323	5349	0.000005	0	--
Triturus macedonicus	0	16569	COX1	5298	6848	0.016	0.204	0.110	1822	0.098129	0.002007	0.0204
Triturus macedonicus	0	16569	COX2	6991	7678	0.221	0.304	0.262	4345	0.082244	0.000008	0.0001
Triturus macedonicus	0	16569	COX3	8594	9377	0.414	0.509	0.462	7647	0.052032	0.001785	0.0343
Triturus macedonicus	0	16569	CYTB	14126	15266	1.082	1.220	1.151	19068	0.108615	0.000011	0.0001
Triturus macedonicus	0	16569	ND1	2702	3668	0.703	0.820	0.761	12616	0.076529	0.004645	0.0607
Triturus macedonicus	0	16569	ND2	3881	4915	0.845	0.970	0.908	15041	0.096995	0.008129	0.0838

Triturus macedonicus	0	16569	ND3	9447	9794	0.517	0.559	0.538	8917	0.024143	0.005917	0.2451
Triturus macedonicus	0	16569	ND4	10152	11529	0.602	0.769	0.685	11356	0.084254	0.010453	0.1241
Triturus macedonicus	0	16569	ND4L	9862	10158	0.567	0.603	0.585	9696	0.116752	0.000012	0.0001
Triturus macedonicus	0	16569	ND5	11738	13552	0.794	1.013	0.903	14966	0.086594	0.008758	0.1011
Triturus macedonicus	0	16569	ND6	13538	14056	1.011	1.074	1.042	17270	0.094335	0.009491	0.1006
Triturus marmoratus	0	16546	ATP6	7915	8598	0.333	0.415	0.374	6189	0.095414	0.005353	0.0001
Triturus marmoratus	0	16546	ATP8	7757	7924	0.314	0.334	0.324	5358	0.059378	0.000006	0.0001
Triturus marmoratus	0	16546	COX1	5298	6848	0.016	0.204	0.110	1822	0.085063	0.001911	0.0225
Triturus marmoratus	0	16546	COX2	6995	7682	0.222	0.305	0.263	4353	0.060361	0.000006	0.0001
Triturus marmoratus	0	16546	COX3	8598	9381	0.415	0.510	0.463	7655	0.095428	0.001752	0.0184
Triturus marmoratus	0	16546	CYTB	14132	15272	1.084	1.222	1.153	19079	0.065388	0.006443	0.0985
Triturus marmoratus	0	16546	ND1	2699	3665	0.702	0.819	0.761	12586	0.063262	0.008082	0.1277
Triturus marmoratus	0	16546	ND2	3879	4917	0.845	0.970	0.908	15018	0.131938	0.01289	0.0977
Triturus marmoratus	0	16546	ND3	9451	9798	0.518	0.560	0.539	8925	0.037173	0.005976	0.1608
Triturus marmoratus	0	16546	ND4	10158	11535	0.604	0.770	0.687	11369	0.077889	0.003743	0.0481
Triturus marmoratus	0	16546	ND4L	9868	10164	0.569	0.605	0.587	9708	0.106771	0.000011	0.0001
Triturus marmoratus	0	16546	ND5	11744	13558	0.796	1.015	0.905	14978	0.079987	0.010145	0.1268
Triturus marmoratus	0	16546	ND6	13544	14062	1.013	1.076	1.045	17282	0.108316	0.004341	0.0401
Triturus pygmaeus	0	16442	ATP6	7914	8597	0.334	0.418	0.376	6181	0.127561	0.011749	0.1244
Triturus pygmaeus	0	16442	ATP8	7756	7923	0.315	0.336	0.325	5349	0.114507	0.014244	0.1244
Triturus pygmaeus	0	16442	COX1	5301	6851	0.017	0.205	0.111	1822	0.075409	0.001009	0.0134
Triturus pygmaeus	0	16442	COX2	6994	7681	0.223	0.306	0.264	4345	0.127503	0.0022	0.0173
Triturus pygmaeus	0	16442	COX3	8597	9380	0.418	0.513	0.465	7647	0.079043	0.001767	0.0224
Triturus pygmaeus	0	16442	CYTB	14129	15269	1.090	1.229	1.160	19068	0.0847	0.002576	0.0304
Triturus pygmaeus	0	16442	ND1	2703	3669	0.701	0.818	0.759	12484	0.089124	0.009548	0.1071
Triturus pygmaeus	0	16442	ND2	3883	4921	0.844	0.970	0.907	14916	0.081517	0.006371	0.0782
Triturus pygmaeus	0	16442	ND3	9450	9797	0.521	0.563	0.542	8916	0.111422	0.005208	0.0467

Triturus pygmaeus	0	16442	ND4	10155	11532	0.607	0.775	0.691	11357	0.094901	0.005007	0.0528
Triturus pygmaeus	0	16442	ND4L	9865	10161	0.572	0.608	0.590	9696	0.029542	0.006085	0.206
Triturus pygmaeus	0	16442	ND5	11741	13555	0.800	1.021	0.910	14966	0.075937	0.004449	0.0586
Triturus pygmaeus	0	16442	ND6	13541	14059	1.019	1.082	1.050	17271	0.097826	0.004364	0.0446
Tylototriton verrucosus	1	17100	ATP6	7922	8605	0.325	0.405	0.365	6247	0.436608	0.031068	0.2425
Tylototriton verrucosus	1	17100	ATP8	7764	7931	0.307	0.326	0.317	5415	0.276997	0.067179	0.2425
Tylototriton verrucosus	1	17100	COX1	5308	6858	0.020	0.201	0.110	1885	0.280186	0.002914	0.0104
Tylototriton verrucosus	1	17100	COX2	7002	7689	0.218	0.298	0.258	4411	0.10353	0.00001	0.0001
Tylototriton verrucosus	1	17100	COX3	8605	9388	0.405	0.497	0.451	7713	0.053741	0.000005	0.0001
Tylototriton verrucosus	1	17100	CYTB	14134	16131	1.052	1.286	1.169	19985	0.09504	0.00873	0.0919
Tylototriton verrucosus	1	17100	ND1	2703	3671	0.715	0.828	0.772	13194	0.308234	0.011686	0.0379
Tylototriton verrucosus	1	17100	ND2	3886	4929	0.853	0.975	0.914	15635	0.473378	0.029314	0.0619
Tylototriton verrucosus	1	17100	ND3	9459	9804	0.505	0.546	0.525	8983	0.323423	0.022529	0.0697
Tylototriton verrucosus	1	17100	ND4	10164	11541	0.588	0.749	0.668	11425	0.43347	0.022726	0.0524
Tylototriton verrucosus	1	17100	ND4L	9874	10170	0.554	0.588	0.571	9764	0.207879	0.019325	0.093
Tylototriton verrucosus	1	17100	ND5	11749	13560	0.773	0.985	0.879	15029	0.325421	0.013863	0.0426
Tylototriton verrucosus	1	17100	ND6	13546	14064	0.983	1.044	1.013	17330	0.032107	0.009792	0.305

Genbank Species	Mitochondrial Gene Order
NC006888 Ambystoma andersoni	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 ND6 Glu CYTB Thr Pro
NC014568 Ambystoma barbouri	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 ND6 Glu CYTB Thr Pro
NC006890 Ambystoma californiense	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 ND6 Glu CYTB Thr Pro
NC006889 Ambystoma dumerilii	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 ND6 Glu CYTB Thr Pro
NC006336 Ambystoma laterale	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 ND6 Glu CYTB Thr Pro
NC005797 Ambystoma mexicanum	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 ND6 Glu CYTB Thr Pro
NC014571 Ambystoma texanum	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 ND6 Glu CYTB Thr Pro
NC006887 Ambystoma tigrinum	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 ND6 Glu CYTB Thr Pro
NC004926 Andrias davidianus	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 ND6 Glu CYTB Thr Pro
NC007446 Andrias japonicus	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 ND6 Glu CYTB Thr Pro
NC006327 Aneides flavipunctatus	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 CYTB Thr ND6 Glu Pro
NC006338 Aneides hardyi	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 CYTB Thr Glu Pro
NC006340 Batrachoseps attenuatus	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 ND6 Glu CYTB Thr Pro
NC006333 Batrachoseps wrighti	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 ND6 Glu CYTB Thr Pro
NC008077 Batrachuperus longidens	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 ND6 Glu CYTB Thr Pro
NC008083 Batrachuperus pinchonii	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 ND6 Glu CYTB Thr Pro
NC008085 Batrachuperus tibetanus	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 ND6 Glu CYTB Thr Pro
NC012430 Batrachuperus yenyuanensis	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 ND6 Glu CYTB Thr Pro
NC006344 Bolitoglossa n. sp.	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 ND6 Glu CYTB Thr Pro
NC006339 Desmognathus fuscus	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 ND6 Glu CYTB Thr Pro
NC006337 Desmognathuswrighti	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 ND6 Glu CYTB Thr Pro
NC017870 Echimotriton andersoni	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 ND6 Glu CYTB Thr Pro
NC006328 Ensatina escholtzscholtzi	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 ND6 Glu CYTB Thr Pro
NC006329 Eurycea bislineata	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 ND6 Glu CYTB Thr Pro
NC006341 Gymrophilus porphyriticus	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 ND6 Glu CYTB Thr Pro
NC006342 Hemidactylum scutatum	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 ND6 Glu CYTB Thr Pro
NC006345 Hydromantes brunus	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 ND6 Glu CYTB Thr Pro
NC008076 Hynobius amiensis	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 ND6 Glu CYTB Thr Pro
NC009335 Hynobius arisanensis	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 ND6 Glu CYTB Thr Pro
NC008088 Hynobius chinensis	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 ND6 Glu CYTB Thr Pro
NC008084 Hynobius formosanus	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 ND6 Glu CYTB Thr Pro
NC013762 Hynobius guabangshaniensis	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 ND6 Glu CYTB Thr Pro
NC008079 Hynobius leechii	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 ND6 Glu CYTB Thr Pro
NC010224 Hynobius queplaensis	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 ND6 Glu CYTB Thr Pro
NC013825 Hynobius yangi	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 ND6 Glu CYTB Thr Pro
NC008078 Liuashishi	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 ND6 Glu CYTB Thr Pro
NC008081 Liua tsinpanensis	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 ND6 Glu CYTB Thr Pro
NC002756 Lyciasalamandra atifi	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 ND6 Glu CYTB Thr Pro
NC006326 Oedipina poelzi	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 ND6 Glu CYTB Thr Pro
NC008089 Onychodactylusfischeri	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 ND6 Glu CYTB Thr Pro
NC008080 Pachyhylobius shangchengensis	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 ND6 Glu CYTB Thr Pro
NC008091 Paradactylyodon gorganensis	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 ND6 Glu CYTB Thr Pro
NC008090 Paradactylyodon mustersi	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 ND6 Glu CYTB Thr Pro
NC006407 Paramesotriton hongkongensis	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 ND6 Glu CYTB Thr Pro
NC006344 Phaeognathus hubrichti	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 ND6 Glu CYTB Thr Pro
NC006343 Plethodon cimerus	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 ND6 Glu CYTB Thr Pro
NC006335 Plethodon elongatus	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 ND6 Glu CYTB Thr Pro
NC006334 Plethodon petraeus	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 ND6 Glu CYTB Thr Pro
NC006332 Pseudoeurycea ruber	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 ND6 Glu CYTB Thr Pro
NC006421 Ranodon sibiricus	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 ND6 Glu CYTB Thr Pro
NC004021 Rhacocotriton variegatus	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 ND6 Glu CYTB Thr Pro
NC008082 Salamandrella keyserlingii	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 ND6 Glu CYTB Thr Pro
NC006325 Stereochilus marginatus	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 ND6 Glu CYTB Thr Pro
NC006336 Thorius n. sp.	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 ND6 Glu CYTB Thr Pro
NC015788 Triturus carnifex	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 ND6 Glu CYTB Thr Pro
NC015790 Triturus cristatus	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 ND6 Glu CYTB Thr Pro
NC015791 Triturus dobrogicus	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 ND6 Glu CYTB Thr Pro
NC015792 Triturus karelinii	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 ND6 Glu CYTB Thr Pro
NC015794 Triturus macedonicus	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 ND6 Glu CYTB Thr Pro
NC015795 Triturus marmoratus	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 ND6 Glu CYTB Thr Pro
NC015796 Triturus pygmaeus	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 ND6 Glu CYTB Thr Pro
NC017871 Tylototriton verrucosus	Phe 12S Val 16S Leu NDI Ile Gln Met ND2 Trp Ala Asn Cys Tyr COX1 Ser Asp COX2 Lys ATP8 ATP6 COX3 Gly ND3 Arg ND4L ND4 His Ser Leu ND5 ND6 Glu CYTB Thr Thr Pro

Figure A2.1 Summary of NCBI Genbank numbers and mitochondrial gene order for 62 salamander mitochondrial genomes analyzed in our study.